THE SUPRARENAL CORTEX

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Edited by

J M YOFFEY

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an enormous number of investigations For the medical man, whether he be a clinician or a laboratory worker, the steroid hormones seem to have opened up entirely new vistas in the study of normal and diseased processes For the organic chemist, the innumerable modifications which can be introduced into the basic 1, 2-cyclopentenophenanthrene structure seem to have provided an extremely rich and fertile field for study For the psychologist the steroid hormones constitute a most potent addition to those endocrine factors which influence mental processes and personality, and which seem indeed to have given to medicine in the twentieth century the basis of a new humoral theory In short, the steroid hormones exert such powerful and widespread effects, that their ramifications extend into almost every part of the body, and influence many of its most important mechanisms

In preparing these proceedings for the press no attempt has been made to conform to the stricter editorial canons, and to introduce uniformity of style, or even of terminology 'Adrenal and Suprarenal to give but one example of many will be found to occur quite indiscriminately, despite the official title of the Symposium As far as possible individual peculiarities of style and expression have been given full play Both in the papers and in the subsequent discussions an effort has been made to preserve something of the friendly and informal atmosphere which constitutes so vital an element in a Symposium of this kind difficult though it may be to translate into print The discussions have not been recorded verbatim but the reports convey with a fair degree of accuracy the gist of what was said

This preface affords perhaps the most suitable opportunity of placing on record my thanks to the many who gave such willing assistance Our Guest of Honour was Sir Henry Dale O M and at the Annual Dinner he discharged with conspicuous success the task of expounding to the members of the Colston Research Society the main features of the Symposium Needless to say, I am also most grateful to all our other invited guests, who contributed to the Symposium a good deal more than their scientific communications To Dr G B Wolstenholme, the Secretary of the Ciba Foundation, I am particularly indebted for making it possible to bring to this country from abroad a number of guest speakers whom we would not otherwise have been able to welcome in our midst

It is a very special pleasure to place on record my debt of gratitude to the Recorders, Dr W K Metcalf and Dr Francis B Robinson, for the indefatigable manner in which they coped with their difficult task My thanks are also due to the Symposium Secretary, Dr E J Field as also to Miss L Lloyd whose secretarial services have helped so much in the preparation of this volume Last but not least, I am deeply indebted to the printers, Messrs J W Arrowsmith Ltd for the unfailing courtesy and efficiency with which they have performed the actual printing of this volume

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Some Aspects of the Preparation and Properties of Adrenocorticotropic Hormone

by

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INTRODUCTION

THE pituitary control of adrenal cortical function had its conclusive demonstration in 1930 by Smith[43] who at the same time provided evidence for the presence of adrenocorticotropic activity in the pituitary gland From that time on the numerous attempts on the part of various investigators notably Collip Anselmino Morris Bates and others to purify and isolate the hormone met with no success until 1942–1943 when two groups of workers [0, 1, 41] published independent announcements of the preparation of highly purified proteins from sheep and pig pituitary glands These proteins were free from other anterior pituitary contaminants and were found to produce selective stimulation of the adrenal cortex of hypophysectomized rats as evidenced both by increase of adrenal weight and by repair of adrenal histology With this the existence of an adrenocorticotropic hormone (ACTH) was definitely established

The first indications from early ultra filtration studies[1, 6, 48] seemed to be that the ACTH activity might be associated with a low molecular weight substance However it has been established recently that ACTH is peptide in nature and that this peptide ACTH is metabolically active in human subjects[3, 4, 5, 9, 19, 2, 5, 27, 30, 3, 34, 50] The fact that partial pepsin digestion of the whole ACTH protein does not destroy *the hormonal activity as first demonstrated by us or that further diges*tion with pepsin [1, 30] does abolish the adrenal stimulating potency provides the best evidence for the existence of an ACTH peptide or peptides

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PROBLEMS OF BIOASSAY

The various methods which have been advanced for the estimation of adrenocorticotropic activity are generally derived from manifestations of either direct or indirect effects of ACTH on normal or hypophysectomized animals. The direct effects are primary, that is they follow directly from the hormone administration without the intervention of adrenal cortical steroids. They include the depletion of ascorbic acid and cholesterol, weight maintenance and hypertrophy of the adrenals and the repair of histological changes due to hypophysectomy. The indirect effects are those which result from the formation and release of the adrenal cortical steroids following ACTH administration; they include eosinopenia, lymphopenia, involution of thymus and lymph nodes, and the like.

For the past few years it has been the ascorbic acid depletion test[4] which has been the most widely employed as an index of ACTH activity. Meanwhile, a number of observations have been made in this and other laboratories which indicate the existence of discrepancies between ACTH potencies as determined by this test and those obtained by various other assay methods. At least a part of the difficulty may be due to the present lack of understanding of the relationship of ascorbic acid content to adrenal function as a whole. It cannot at all be assumed that the correlation is a simple and direct one; for example, Jailer and Boas[15] found that although chronic administration of ACTH to the young chick caused adrenal hypertrophy no alteration of the adrenal ascorbic acid content was observed. Moreover, by the administration of ACTH it was possible to induce in the scorbutic guinea pig an increase of adrenal activity[13, 36, 49] from which it appears evident that ascorbic acid is not needed for the production and secretion of the corticosteroids which form as the result of ACTH injections. In view of all this, the adrenal ascorbic acid depleting test would appear to be an inadequate measurement of the various types of stimulation of the adrenal cortex by ACTH. Hence, it is unfortunate that the Committee on Biological Standardization of the World Health Organization has adopted the adrenal ascorbic acid depleting test as the official assay method for determining ACTH activity, neglecting the importance of other assay techniques.

The original observations of Li and Greenspan[24] that no direct correlation between the adrenal ascorbic acid (AA) depleting and adrenal weight (AW) maintenance activities exists among various ACTH preparations, has now been confirmed by a number of investigators[8, 35, 39, 52]. In fact, Dixon *et al*[7] claimed that the AW factor could be separated from the AA factor in a pig ACTH preparation by means of ion exchange chromatography. A comparison of the potencies of different ACTH preparations, as determined by the ascorbic acid assay and the cholesterol depletion test, also indicated no correlation between these two tests[51]. This lack of correlation is more strikingly emphasized by Oesterling and Long[36] who observed that despite the fact that there is practically no ascorbic acid in the scorbutic guinea pig adrenal definite adrenal cholesterol depletion took place after ACTH stimulation.

Further, Hungerford *et al*[1] have presented a detailed account of the influence of whole ACTH protein preparations, and their partial hydrolysates as obtained by pepsin or acid, on lymphoid tissues and thoracic duct lymphocytes. The results showed clearly that some preparations were active in causing a thoracic duct

lymphopenia and thymic involution whereas other preparations produced no such effect.

The observation that there was no obvious correlation between the ascorbic acid activity of various ACTH preparations and the degree of eosinophil change in normal mice[38] has been extended to an investigation of the eosinopenic effect of ACTH in adult male rats[11]. The ascorbic acid depleting activity of the four ACTH preparations employed were respectively 0.2, 2, 10 and 50 times as active as the International Standard. At the 0.5 mg dose level these preparations exhibited eosinophil depressions of respectively, −74 per cent, −81 per cent, −88 per cent and −59 per cent with the saline control showing a depletion of −17 per cent. These same ACTH preparations were given at 0.25 and 1.0 mg dose levels and showed the same relative degree of eosinophil depression. These results give further corroboration to the conclusion that the factor which depletes adrenal ascorbic acid is not directly correlated with the ACTH factor which produced in these experiments a blood eosinopenia.

A similar discrepancy was observed between the metabolic effects of an ACTH protein and a pepsin peptide given subcutaneously by continuous injection to normal male rats. Ingle and Li[14] observed that the ACTH protein was more effective than was the ACTH peptide in causing signs of hypercorticalism (glucosuria, rise in urinary nitrogen, and loss of body weight). On the other hand the ACTH peptide had 2–3 times the biological activity of the ACTH protein when measured by the ascorbic acid depleting test.

It is apparent from these data that the lack of correlation between the results derived from the ascorbic acid depleting test and from various other criteria of adrenocorticotropic activity can now be said to be established. In the interpretation of these results the existence of more than one ACTH factor may easily be assumed but before such a conclusion is unequivocally accepted the following considerations should be kept in mind: the variations in the route of administration in applying the different tests, difference in the rate of absorption of material prepared by different chemical techniques and the importance of differential time response relationships between different biological phenomena which may not always be assumed to bear causal or dependent relationships.

Recently Johnsson and Hogberg[16] and Sulman[4, 48] have claimed that intermedin and ACTH were closely related or identical. Moreover they have suggested that the assay for the melanophore expansion activity in the frog was a rapid and simple method for testing for adrenocorticotropic hormone. However our data[40] are in disagreement with the claims of these investigators. When a number of sheep ACTH preparations having activities of from 30–100 USP units/mg, as based upon the adrenal ascorbic acid depleting activity, were also assayed for melanophore expansion potency using hypophysectomized *Rana pipiens*, no positive correlation was observed between the two tests; in fact those preparations tested showed an inverse correlation. In addition activation of intermedin activity by treatment with 0.1 N NaOH at 100° for five minutes[41] was found to decrease the ascorbic acid depleting activity to one twentieth of its original value. Furthermore separation of intermedin and ACTH was achieved by the use of a discontinuous pH gradient on oxycellulose[10] and by zone electrophoresis on paper[18]. A rather surprising result was

obtained when anterior and intermediate lobes of frog pituitary were assayed for each activity, although melanophore expansion potency in the *pars intermedia* was thirty times greater than that in the *pars distalis*, the ACTH activity in the *pars intermedia* was about one half that of the anterior lobe

PREPARATION OF SHEEP ACTH PREPARATION E

Payne, Raben and Astwood[37] have given details of methods for the preparation of highly active ACTH fractions from acetone dried pig pituitaries The procedure which uses acetic acid for the extraction is similar to that of Kamm *et al* [17] for the fractionation of posterior pituitary extracts, the method entailing an initial extraction with glacial acetic acid at 70° and subsequent fractionation with acetone and ether The active material is adsorbed on powdered cellulose and eluted with hydrochloric acid Later Astwood *et al* [2] obtained a twenty to eighty fold purification by adsorption and elution from oxycellulose A combination of glacial acetic acid extraction of the acetone dried *pig* anterior pituitaries and oxycellulose adsorption yields possibly the best simple method for the preparation of highly active material in maximal yield

A simple procedure for the preparation of a biologically purified and clinically active ACTH in good yield from sheep, pig and horse pituitaries has been described[26, 28] A combination of this procedure with the oxycellulose procedure of Astwood *et al* provides an effective method for obtaining highly active ACTH preparations[23, 9]

One kilogram of frozen whole sheep pituitary glands was finely ground and extracted with 4 1 litres of acid acetone solution (0 5 litre of water 4 litres of acetone and 100 cc concentrated HCl) by vigorous stirring for one hour The mixture was filtered and the residue re extracted with 2 litres of 80 per cent acetone After removal of the residue by filtration, the combined extracts were poured into 30 litres of cold acetone The precipitate formed was dried *in vacuo* after repeated washing with acetone The product resulting from this procedure is designated as acid acetone powder (AAP) and the yield from one kg of glands averages 35 gms

Twenty grams of AAP was next dissolved in 940 cc water and adjusted to pH 3 0 A saturated NaCl solution (60 cc) was added dropwise with constant stirring the precipitate formed was centrifuged off and saved for the isolation of lactogenic hormone The supernatant was brought to saturation and dissolved in 100 cc water and dialysed against running water until salt free The dialysed solution was frozen and dried in vacuum the yield being 4 grms The resulting purified ACTH protein was designated as Preparation D

The next step adsorption on oxycellulose is essentially the same as the procedure described by Astwood *et al* [2] Preparation D (8 gms) was dissolved in 250 cc 0 1 M acetic acid, the pH of the solution was between 3 5 and 4 0 One gram of oxycellulose (10–12 per cent COOH) was added, after the mixture had been stirred at room temperature for thirty six hours it was kept for a few hours at 0° C Most of the clear supernatant was decanted off, the residue was washed twice with distilled water and then extracted with 100 cc 0 1 M HCl by constant stirring at room temperature for twelve hours The mixture was filtered and the filtrate dialysed against distilled

water until it was free of HCl Lyophilization of the dialysed solution yielded 20 mg of a white powder having a potency of 50–100 USP Units/mg as assayed by the adrenal ascorbic acid depleting method This material is called Preparation E

By this method a virtually complete recovery of ACTH is achieved The potency is increased from 0 6 to 60 USP Units/mg and is comparable to that of the material prepared by Astwood *et al* The product also exhibits activity in maintaining the adrenal weight of hypophysectomized rats although its potency in adrenal weight maintenance appears to be lower than its adrenal ascorbic acid depleting activity In addition this product has comparatively low eosinopenic activity in normal adult rats

PURIFICATION OF PREPARATION E

The physicochemical properties of Preparation E are markedly different from those of the ACTH protein previously obtained Tryptophane and tyrosine are present in the amount of 3 per cent and 5 per cent respectively, the tyrosine being double the amount contained in the ACTH protein there was 0 7 per cent which can be accounted for by the value of methionine Ultracentrifuge studies obtained a sedimentation constant S_0 of 0 8 S for Preparation E and its average molecular weight is assumed to be in the neighbourhood of 10 000 After studies on paper electrophoresis at various pH it was concluded that the isoelectric point of the component with which the ACTH activity is associated is located in the neighbourhood of pH 9 indicating the basic nature of the hormone Partial hydrolysis with pepsin and acid causes no loss of ACTH activity and acid treatment even appeared to enhance the potency The latter observation seem to confirm the earlier report[23] to this effect

Further purification of Preparation E can be effected by the following procedures

Trichloroacetic Acid (TCA) Fractionation 100 mg of Preparation E was dissolved in 5 cc H_2O and made up to 5 per cent TCA by adding an equal volume of 10 per cent TCA solution the resulting precipitate was re dissolved in 5 cc H_2O and the 5 per cent TCA precipitation was repeated four times The final precipitate was dissolved in water dialysed and lyophilized the product was designated as Preparation EP The combined 5 per cent TCA soluble supernatants were mixed with an equal volume of 50 per cent TCA solution It was found that the 25 per cent TCA soluble fraction was devoid of ascorbic acid depleting activity whereas the 25 per cent TCA precipitate contained all the hormone potency The 25 per cent TCA precipitate was next dissolved in water and passed through an IR–4B column as previously described[30] to remove the TCA The resulting TCA free solution was then lyophilized this product is designated as Preparation ES The ascorbic acid depleting potency of Preparation EP was found to be significantly lower than that of Preparation ES The specific activity of Preparation ES is about 80 USP Units/mg in contrast to the potency of the starting material which is 30 USP Units/mg

Dioxane Fractionation It was observed that Preparation E is soluble in acidified 50 per cent dioxane solution but becomes partially precipitable at pH 9 3–9 4 Results of many experiments showed that 60 per cent of the material which is soluble in 50 per cent dioxane at pH 9 3–9 4 contains most of the ACTH activity By such a simple procedure a two fold purification can easily be obtained It may

be pointed out that there occurred no loss of activity even when this solvent was brought to such a high pH

Cellulose Column Chromatography We have recently described a procedure using cellulose oxycellulose to achieve a two to four fold purification of Preparation E[19], working under *non equilibrium* conditions In a typical experiment 10 mg of Preparation E was pre sed into a column (7 × 115 mm) containing a mixture of 200 mg oxycellulose and 600 mg cellulose powder (Solka Floc), washed according to the procedure of Astwood *et al* [26] Development was accomplished by means of a discontinuous pH gradient with 40 ml 0 3 N $ClCH_2COOH$, followed by 40 ml 0 7 N $ClCH_2COOH$, and finally by 20 ml 0 1 N HCl The flow rate was maintained at 4–1 ml per hour Analysis of each fraction by the method of Lowry *et al* [31] revealed three peaks whose average distribution of material is 41 per cent in the first, 31 per cent in the second and 28 per cent in the third the ACTH activity was found to be concentrated almost completely in the second peak Thu , fractions possessing a potency of approximately 120–200 USP Units/mg may be obtained

When the purification procedure was followed by filter paper electrophoresis[18] it was demonstrated that at pH 8 the first peak contained all of the anionic component(s) and the last peak the strongly cationic component(s) The middle peak showed a distribution of material at the origin or near it on the cathode side areas where the activity had been found previously under these conditions[23 29]

Displacement Chromatography Attempts to apply the displacement technique of Tiselius[47] to the fractionation of Preparation E have met with many difficulties Since the technique had been shown to be useful for the purification of ACTH peptide mixtures[27] it was decided that it might be workable to submit Preparation E to partial hydrolysis before it is applied to the column The following conditions were developed by J O Porath in this laboratory for this purpose

The adsorbent was prepared as previously described[27] the sections of the columns had the following volumes 14, 6 5 5, 3 5 and 3 5 ml The adsorbent in the column was pre treated with 0 5 per cent octanol 10 mg of a peptic digest of Preparation E[23 9] were dissolved in 1 cc 0 5 per cent octanol and applied to the column It was then eluted with 75 cc 0 5 per cent octanol and followed by 50 cc 2 4 per cent decanol as the displacer Fifty per cent ethanol containing 0 1 M HCl was used as the solvent for the higher alcohols Each fraction (3 5 cc) was collected in an automatic fraction collector an aliquot of each fraction was employed for ninhydrin colour determination [33] Typical experiments revealed the elution fraction to be devoid of ACTH activity while the displacement fraction contains almost all the ACTH potency evincing a three fold purification

COMMENTS

Although full characterization of ACTH will have to wait upon its isolation in pure form there is little doubt that the hormone is a peptide with an isoelectric point in the neighbourhood of pH 9 It is difficult to predict the potency of the pure product but we believe that the final purified material is likely to possess a potency higher than 400 USP Units/mg The following amino acids have been found to be absent from the purest preparations hitherto obtained in this laboratory cystine,

methionine tyrosine serine threonine, isoleucine, and histidine The hormonal activity is easily destroyed by oxidation and is remarkably stable in acid solution The observation that partial acid hydrolysis of ACTH preparations sometimes causes an enhancement of ascorbic acid depleting activity is still difficult to explain Reactions with ketene nitrous acid and acetic anhydride indicate that the free amino groups are essential for the hormone activity

The absence of correlation between the ascorbic acid depleting activity and other indices of hyperadrenocorticism in animals resulting from the administration of various ACTH fractions may suggest the existence of more than one adrenal stimulating hormone However, as it has already been pointed out final acceptance of the existence of multiple ACTH factors must wait upon further experimental investigation It has been demonstrated that partial hydrolysis of the *whole* ACTH protein preparation with acid not only does not damage but sometimes enhances the ascorbic acid depleting potency whereas it invariably causes a lowering of adrenal weight increasing activity (AWF) with a corresponding decrease in ability to inhibit body growth to depress lymphoid tissues, and to cause eosinopenia and thoracic duct lymphopenia It is notable that Preparation E has a low potency with regard to its effect in causing an eosinopenia in normal rats despite its high ascorbic acid depleting activity while on the contrary some fractions possess good eosinopenic effects but low ascorbic acid depleting activities The question of whether or not there exists a separate eosinopenic factor (EF) in the *whole* ACTH molecule remains to be investigated

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The Preparation and Properties of ACTH

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LIKE Dr C H Li we are uncertain what is the significance of the fall of the ascorbic acid content of the adrenal glands of hypophysectomized rats treated with ACTH but unlike Dr Li we hope it means something We have, in fact set out to make preparations of the factor concerned with the hope that ultimately it can be isolated and perhaps synthesized It seems reasonable to call this factor and other factors which act on the adrenal gland ACTH and this is the term that we shall use Until ACTH has been isolated and the active substance completely characterized, we can talk with no certainty about the properties of the active material But in the meantime we can at least say something about the properties of substances which are not ACTH and can also hazard a few guesses about some of the properties which the active material may possess

Li and his collaborators showed some years ago that ACTH activity survived in part at least the treatment of so called protein hormone with pepsin or acid under defined conditions this led to an intensive search for synthesizable peptides with ACTH activity In an attempt to fractionate a mixture of peptides produced by acid hydrolysis by what may be called *orthodox methods* it was observed (in part in collaboration with Dr H G Khorana of the Department of Chemistry University of Cambridge) that ACTH activity moved to the cathode in an electrodialytic cell that its movements in aqueous phenol chromatograms were retarded in an acid atmosphere and that when ACTH active material was partitioned between lipid and aqueous solvents the partition ratio was altered in favour of the aqueous phase in the presence of hydrochloric acid These various observations suggested the possibility that the active material might be basic in character It was just about this time that the results of Payne Raben and Astwood (1950) were published According to these authors ACTH activity could be adsorbed onto powdered cellulose from dilute acetic acid solution and eluted again with 0 1 N HCl Such behaviour agreed also with the postulate that the active factor might be basic in character

The basic proteins lysozyme and ribonuclease had been isolated by means of resolution on a column of an ion exchange resin and since Dr Stanford Moore who had experience in this type of work was in Cambridge at the time when it was first considered that ACTH might be basic in nature he collaborated with us in the application of acidic ion exchange resins to the purification of ACTH We have largely used Amberlite IRC–50 a polymer of methacrylic acid cross linked with divinyl benzene In experiments involving flow through columns of ion exchange resins the separation of substances will be expected to depend upon differences in

acid base properties Almost immediately after adapting ion exchange technique to the ACTH problem, we were able to separate from the so called protein hormone a fraction which contained most of the biological activity in a portion yielding only 5 per cent of the total ninhydrin colour (Dixon, Moore Stack Dunne and Young 1951) I should emphasize that this separation of the activity from the so called protein hormone had been effected on material which had been subjected to no obviously hydrolytic step and it could hardly be accepted that interaction with a carboxylic ion exchange resin brought about hydrolysis It therefore seemed that a small fraction, pre existing in a separable form was present in the protein hormone and that no hydrolysis was needed to separate it from the protein with which it was associated It perhaps should be pointed out that the method of extracting ACTH activity as a preliminary step in the preparation of the protein hormone involves the use of acid acetone and it was possible that some hydrolysis had occurred during the early stages of the preparation of the protein hormone It also seemed possible that the protein hormone was itself of no native significance

While this work was in progress Astwood, Raben Payne and Grady (1951) de scribed a single step adsorption of ACTH activity from 0 1 N acetic acid on oxy-cellulose (10 4 per cent COOH) with subsequent elution by 0 1 N HCl, which would again agree with the basic properties of the active material

The mechanism of action of either the oxycellulose or the ion exchange resin is perhaps uncertain With respect to the ion exchange resin it may well be that at pHs near 7 ACTH is positively charged, and is therefore held back by the negatively charged resin possibly because of the attraction of the negatively charged carboxyl groups for the positive basic groups of ACTH, and also because the net charge of the whole molecule is positive At higher pH values, for instance in the presence of a suitable sodium phosphate buffer ACTH becomes less positively or even nega tively charged, and is held by the resin much less strongly In such a buffer there is also an excess of sodium ions to displace the ACTH active material from the resin Thus raising the pH increases the rate at which ACTH active material moves down a column packed with the resin We have found by experiment that pHs be tween 6 6 and 6 8 are suitable for retarding ACTH active material and for allow ing much of the inactive protein substance which is more acidic and so less positively charged at this pH to move faster down the column and thus to be separated from the ACTH activity It is clear that interaction of ACTH active material with the carboxyl groups of oxycellulose may also follow such a course

But it is not certain that interaction with the carboxyl groups is the only important consideration In fact it may well be that particularly with oxycellulose some effect other than interaction with the carboxyl groups is highly significant since with oxy-cellulose in acid medium equilibrium is only slowly obtained Presumably it is because equilibrium conditions are rapidly obtained with the system that we have used that we have been able to resolve a complex mixture into separable fractions by a relatively rapid flow through a column of the ion exchange resin

One problem that arises when ion exchange resins are used for the fractionation of ACTH activity is the removal of the buffering salt from the fractions that are obtained In order to do this we adjust the active material to pH 4 pass the fractions through a column of resin in the acid form and then wash the column with dilute

acetic acid Under these conditions the ACTH is firmly held while sodium ions and unretarded phosphate ions are washed through the column The column is then washed through with normal ammonia, and this elutes the ACTH active material at the front The ACTH activity is now in solution with only ammonia and some ammonium acetate and since the latter is volatile it, together with the ammonia, can be removed in the process of freeze drying In this way the active material is obtained free from salts

In many of our experiments we have used, as a starting material for further purification the pituitary extract made by the method of Payne Raben and Astwood (1950) which involves extraction of the acetone dried pituitary gland with glacial acetic acid acetone mixture at 70° C followed by acetone and ether precipitation Material prepared by Astwood and his colleagues by this method usually has a potency of 1–2 times the international standard, but in our hands we rarely obtain a potency of greater than one times the international standard By passing material

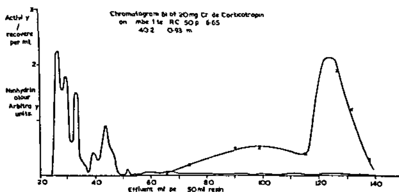


Figure 1

extracted in this way through resolving columns of Amberlite IRC–50 we are able to separate most of the ACTH activity from the protein material (Fig 1 cf Dixon Moore Stack Dunne and Young 1951) As will be seen from Figure 1, under these conditions ACTH activity is spread over two zones while a third is also observed There is a sharp peak of activity well separated from the bulk of the protein and clearly observable on the chromatogram (Fig 1) there is a zone of activity lying between this peak and the main protein fraction and there is a portion of the active material which remains on the column under these conditions but is removable by washing the resin with alkali It will be observed that in Figure 1 there is no obvious peak in the ninhydrin reaction curve (cf Moore and Stein 1951) at the point where the peak of the activity is found but when more concentrated preparations are used it is always the case that a peak in the activity curve is associated with some peak in the ninhydrin reaction curve

When an oxycellulose concentrate of crude corticotropin prepared in our laboratory according to the method of Astwood and his colleagues was passed through a column of Amberlite IRC–50 the results were essentially similar to those we obtained

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activity and that these represent naturally occurring entities though of course it is not necessary to assume that all substances which naturally occur in the gland are secreted into the blood stream

Nearly a year ago we put forward the view (Stack Dunne and Young 1951) that there are at least two factors which should properly be included under the term ACTH—one concerned with the reduction of ascorbic acid in the adrenal of the hypophysectomized rat, while the other is associated with ability to increase the

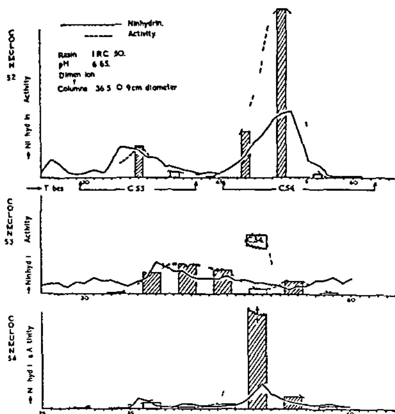


Figure 3 Fractions 30 42 and 46 62 from column 52 as shown were freed from salt and freeze dried The products were run on columns of the same dimensions and under the same conditions 53 and 54 respectively

adrenal weight of the hypophysectomized rat We are of the opinion that any pituitary factor which can increase the weight of the adrenal gland of the hypophysectomized rat has claims to be considered as an adrenocorticotropic hormone We have discussed elsewhere the possibility that the different effects might merely be ascribable to differences in rate of absorption of fractions from the peritoneal cavity (Dixon Stack Dunne Young and Cater 1951) and do not wish to labour this point again More recently it has been observed that two fractions which differ in adrenal weight

with our own fractions (Fig 2) In the two chromatograms shown in Figure 2 the similarity of the ninhydrin peaks in the two different experiments is evident

In order to determine whether the three fractions we obtained would behave consistently on rechromatography, in one instance we separated the two fractions in the effluent, desalted them and then passed them through other Amberlite IRC-50 columns at the same pH (6 65) We found that the position in the effluent at which these fractions emerged was now very similar to that which they had originally occupied (Fig 3), and similarly the ninhydrin reactive components were still associated with the active peaks Likewise, when the fraction which was not eluted from the column by the buffer of 6 65 under these conditions but which was removed from

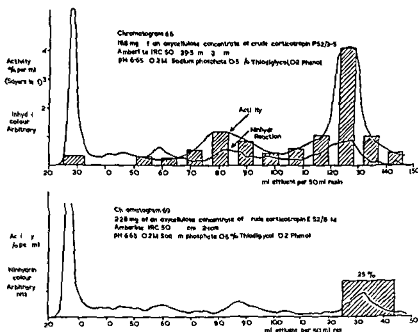


Figure 2

the resin by dilute alkali was passed through a second column in buffer at pH 6 65 it again remained on the column and was again eluted by alkali

At present the interpretation of all these results is not by any means certain It seems likely that the different fractions have different basicities, and it would be conceivable for instance that ACTH is a basic substance and that the fraction which remains on the column and which is the most basic of the three we are considering contains the substance which we are looking for while the two fractions which we obtained in the effluent represent complexes between the basic substance and other materials of a less basic character Clearly it is also possible that ACTH is an acidic substance associated with varying amounts of basic carrier, and a large number of interpretations of this sort might be considered We do not exclude the possibility that indeed there are a number of different substances with ACTH

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and ascorbic acid activities still retain their differences in this respect when they are administered as a suspension in 5 per cent beeswax arachis oil, according to the method described by Bruce and Parkes (1952) Under these conditions the adrenal weight increasing activity was twenty times greater than when the material was injected without the suspending medium, but the difference between the two fractions with respect to relative activities in the adrenal weight increasing test and the ascorbic acid reducing test remained We feel that the simplest interpretation of these results at present is the postulate that different adrenal weight increasing and ascorbic acid reducing factors exist Recently Dr Li and his colleagues (Reinhardt, Geschwind and Li 1951) have discussed the evidence that a multiplicity of adrenocorticotropic hormones exist and their conclusion is that 'the available data are insufficient to affirm or deny that the responses produced by ACTH preparations are the result of multiple ACTH hormones' I am sure my old friend, Dr Li will not mind if I say that this conclusion is a skilful example of sitting on the fence We ourselves find the top of the fence too narrow for comfort, and prefer to attempt to obtain a broader seat even though the passage of time may show that the seat is liable to slip We wish to emphasize that although we believe that the evidence is not yet final and conclusive the experimental findings already to hand appear to us to be most simply interpreted on the hypothesis that there are at least two different factors which influence the adrenal gland of the hypophysectomized rat one which is capable of causing a reduction of ascorbic acid content, while the other increases the weight of the gland It would be surprising if the ascorbic acid reducing factor did not itself exert some action in increasing the weight of the adrenal gland since any substance which increases the metabolic activity of an organ might be expected to bring about some degree of hypertrophy It is perhaps useful at the present stage to point out that as Cater and Stack Dunne (1952) have recently demonstrated adrenal weight increasing pituitary fractions stimulate mitotic activity in the adrenal cortex, where is ascorbic acid reducing fractions even when absorption is delayed show only feeble activity in this respect This difference may well be of substantial significance

If we may now return to the question of the ascorbic acid reducing substance it will be seen that the evidence that we have obtained together with that published by Astwood and his colleagues and by Morris and his co workers points to the conclusion that the active substance is a small fraction of the total protein present in crude extracts, of a biological activity much greater than that of the so called protein hormone Much of the evidence concerning the properties of the active substance is most easily interpreted on the view that it is basic in character

We wish to thank the Trustees of the Nuffield Foundation for a grant from the Oliver Bird Fund which has made this research possible

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Purification of ACTH

by

M L DEDMAN, T H FARMER P MORRIS and C J O R MORRIS

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THE present paper will discuss some methods examined in our laboratory for the purification of the adrenal ascorbic acid depleting factor of the anterior pituitary gland The question of the existence of different biological effects exerted by the anterior pituitary on the adrenal cortex possibly *via* different hormones can only be solved by isolation of the factors concerned and we do not feel that the evidence at present available enables a decision to be made on this matter The converse of the question, the existence of several chemical species with identical biological actions must also await further progress in isolation The whole question is complicated by the marked tendency of the adrenal ascorbic acid depleting factor to form stable complexes with a wide variety of substances Such complexes may and indeed probably will show biological effects differing qualitatively as well as quantitatively from the free substance

The work to be described has been carried out almost entirely with extracts of pig anterior lobes and biological activities assessed by the method of Sayers Sayers and Woodbury (1948)

EXTRACTION

Our previous experience with the solubility of beef ACTH in glacial acetic acid led us to apply a similar method to pig glands Pig anterior pituitary powder pre pared by acetone dehydration contains in our experience 0 23–0 3 I U ACTH/mg Sayers *et al* have found similar values while Raben Payne and Astwood have found somewhat higher values up to 0 4 I U /mg Such acetone dehydrated powders appear to be stable for at least a year We have examined the extraction of ACTH from such powder by acetic acid of varying water content The extraction procedure involved thorough homogenization of the pituitary powder with a twenty fold weight of acetic acid in a high speed macerator for ten minutes The mixture is then shaken mechanically for one hour at room temperature and centrifuged The solid is then re extracted with a further ten fold weight of acetic acid the mixture is shaken for thirty minutes and again centrifuged During the whole process care is taken to exclude atmospheric moisture The combined extracts are then carefully treated with a two fold volume of peroxide free ethyl ether allowed to stand for thirty minutes at room temperature centrifuged and washed very thoroughly twice with ether twice with acetone and finally dried *in vacuo*

The results of these experiments are given in Fig 1

recent work of Ghosh Smith and Sayers (1952) indicates however that this is not the only effect, as ACTH which had been heated in 1N HCl at 100° C still showed marked lability near pH 7 0 The marked instability of even highly purified preparations still constitutes one of the major difficulties in the chemistry of ACTH

To sum up it may be said that the glacial acetic acid extraction method offers a quick and convenient *preparation of ACTH of reasonable potency in high yield* Our own studies with beef pituitary glands indicate that in this case it is far superior to the acid acetone or acid ethanol extraction processes, giving four or five times the yield of biological activity as assayed by the Sayers method

ULTRA FILTRATION

The success of this method applied to beef pituitary extracts (Cortis Jones *et al* 1950) led us to examine the ultra filtration of pig extracts prepared by the glacial acetic acid ether process The results were surprisingly different About 65 per cent of the biological activity was ultra filterable even at neutrality in contrast to the acid *ethanol beef extracts which were ultra filterable only near pH 2 About 60 per cent* by weight of the acetic acid ether fraction was soluble in water and the whole of the water soluble activity was ultra filterable It proved very difficult to ultra filter the remaining 40 per cent of the activity but suitable conditions were finally found The acetic acid ether precipitate was dissolved in 0 2 N formic acid and the ultra filtration carried out through cellophane 300 membranes at 40 C Under these conditions 90–100 per cent of the biological activity passed through the membrane accompanied by 20–30 per cent of the weight The formic acid was removed from the ultra filtrate by exchange against acetic acid using the anion exchange resins Amberlites IR 4 B or IRA 400 in the acetate form The resulting solutions could be lyophilized without loss of potency yielding a white powder assaying 5–7 I U /mg The above results apply only to glacial acetic acid ether fractions which have not been heated during the preparation If such preparations have undergone the short heating at 100 C mentioned earlier only about 30 per cent of the biological activity is ultra filterable even in formic acid at 40 C It is evident that in addition to destroying the proteolytic enzymes heating brings about some change in the accompanying proteins which makes it more difficult to separate the smaller molecular ACTH from them It is also interesting to note that ultra filtration of acetic acid ether fractions in HCl is very inefficient, only about 40 per cent of the activity being ultra filterable The process is thus worse than ultra filtration in water

The specific effect of acetic and formic acids in facilitating separation of ACTH both in the initial extraction and in ultra filtration methods is probably to be explained by the specific dissociating effects of these acids on proteins Bamford Hanby and Happey (1949) and their co workers of the Courtauld Research Group in Maidenhead have shown by X ray diffraction and infra red studies that relatively small amounts of these acids produce remarkable changes in the structures of synthetic amino acid polymers promoting an α keratin β keratin type of transformation Such a change would undoubtedly induce an uncoiling of protein chains especially if we accept the 3 7 residue per turn helix model for protein structures recently suggested by Pauling and co workers (1951) Also anomalously low molecular

It will be seen that while the biological activity is extracted almost quantitatively by 99 75 per cent acetic acid, a further increase of water content does not increase the yield, the total solids extracted increasing steadily with increasing water content When dealing with freshly prepared anterior pituitary powder, which always retains a low but definite water content, *99 75 per cent acetic acid is the extraction medium* of choice If however the powder has been thoroughly desiccated 99 5 per cent acetic acid is better

Preparations made in this way average 1–1 5 I U /mg, although values up to 2 8 I U /m g have occasionally been encountered Such preparations are fairly stable if kept under anhydrous conditions The stability is however greatly improved

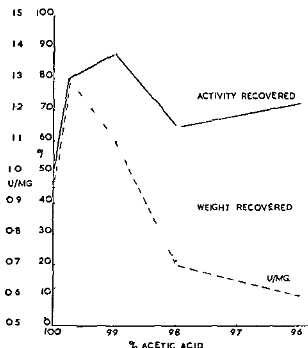


Figure 1

if the extract is heated at 100° C for fifteen minutes prior to the ether precipitation The biological activity is completely unchanged by this process but the chemical properties are somewhat altered as will be seen later

Payne Raben and Astwood (1950) have described a similar process in which the pituitary powder is extracted with 99–99 5 per cent acetic acid at 70° C fractionally precipitated with acetone to remove inactive material and finally precipitated with ether Their material averages 2 I U /mg and appears to be very stable

It appears likely from the findings of Adams and Smith (1951) that a great deal of the instability of ACTH in crude preparations is due to the presence of proteinases in relatively large amount in the pituitary itself The stabilizing effect of heat treatment noted above is thus probably due to the inactivation of these enzymes The

(2) Differences in net ionic mobility under the conditions used Application of the fundamental moving boundary equation to the determination of the rate of elimination of a given ion constituent from the centre compartment shows that the rate is exponential so that the constituent is removed completely only after an infinite time Thus the only possibility of fractionation by differences in net ion mobility in a three compartment cell is to take a series of fractions after definite time intervals from the commencement of the electrodialysis Since actual separation takes place only within the membrane itself the process is in many ways analogous to chromatography the electric current replacing hydrostatic flow As the membrane is in general very thin the resolving power of the method cannot be expected to be very high The choice of medium for the separation is important Since inorganic ions have net mobilities at least ten times larger than most organic ions it is impossible to keep pH and ionic strength constant in the system during the period necessary for separation From this point of view, weak electrolytes have many advantages as electrodialysis mediums The conductivity is low and remains fairly constant throughout the apparatus so that excessive drops in potential do not occur across any one compartment In consequence of the low conductivity heating effects are also small As the net ion mobilities in the medium are of the same order as those of the ions being separated marked pH changes do not occur during the period of separation

From these considerations and the especially favourable properties mentioned before we have used aqueous acetic acid as medium This has also the advantage of volatility so that the separated fractions can easily be recovered by lyophile drying

In order to make maximum use of the molecular sieve effect (1 above) cellophane 300 was employed for both anode and cathode membranes Since cellophane is negatively charged in aqueous media electrendosmotic flow will take place towards the cathode At the low pHs employed this is not however a serious disadvantage

Under the conditions used all the constituents of the ACTH mixture will carry a net positive charge and will move towards the cathode The negatively charged cellophane cathode membrane will thus offer little electrostatic resistance to ions capable of passing through it The use of a positively charged cathode membrane was also considered as the increased resistance to cations due to electrostatic forces might confer increased selectivity Subsequent work however showed that this was impracticable

Preliminary experiments were carried out using acetic acid ether fractions dissolved in 2N acetic acid It was found that the biological activity moved to the cathode compartment under these conditions and loss by electrode reactions was inconsiderable The rate of movement which was at first rapid soon slowed down markedly so that only about 30 per cent of the total activity could be recovered from the cathode compartment the remainder could be recovered from the centre compartment This effect was soon traced to coating of the cathode membrane with a thin layer of protein which although positively charged was unable to traverse the membrane The latter thus became positively charged and was thus relatively impermeable to cations The potency of the material passing through in the early stages of the process was rather lower than that obtained by ultra filtration in formic acid about 3–5 I U /mg Experiments were therefore carried out on ultra-filterable ACTH fractions obtained as described earlier In a representative experiment the

weights for synthetic amino acid polymers in formic acid have been observed by Katchalski *and co workers (1951)* It is perhaps a fortunate coincidence that the Van Slyke amino nitrogen determination is usually carried out in strong acetic acid solutions

Ultra filtration is thus a relatively simple method of preparing ACTH fractions of 4–7 I U /mg The relatively gentle procedures and high activity yield make it unlikely that such products are formed by hydrolysis of a parent molecule, especially as hydrochloric acid is a less efficient ultra filtration medium than acetic and formic acids or even than water

Representative data on ultra-filtrates prepared in different ways are collected in Table I

TABLE I

Ultra filtration of ACTH

Material *Ultra filtered*	Ultra filtration *Medium*	Percentage Activity *in U F*	Percentage Weight *in U F*	U/mg
(1) acetic ether precipitate	2 N acetic acid	60	27	4 0
(2) acetic ether ppt	2 N acetic acid heated	21	33	1 05
(3) acetic ether ppt heated	2 N acetic acid	47	34	2 0
(4) acetic ether ppt heated	0 2 N formic acid	43	18 3	3 4
(5) acetic ether ppt	0 2 N formic acid 40° C	98	22	7 2
(6) acetic ether ppt	2 N propionic acid	34	27	2 0
(7) acetic ether ppt	0 1 N HCl 40° C	40	20	2 0
(8) acetic ether ppt	H O	53	13	4 0

ELECTRODIALYSIS

In view of the valuable separation from material of high molecular weight achieved by ultra filtration we have also examined electrodialysis as a separation method for ACTH using the conventional three compartment cell It is important to be clear as to the nature of the separations possible with this apparatus Separations of substances of similar charge can take place by two mechanisms

(1) Differences in molecular size so that some components will be unable to pass through the membrane Separations involving this factor will be similar to those brought about by ultra filtration

activity could be precipitated almost completely from solution but little or no concentration was achieved Trichloracetic acid proved to be an exception Table III shows that trichloracetic acid present at a final concentration of 15 per cent precipitates the activity quantitatively from solution, precipitation being less complete at lower concentrations and if anything even less complete at higher concentrations At the optimum concentration of trichloracetic acid about 80 per cent of the biological activity together with 20 per cent of the weight was precipitated resulting in a four fold concentration Experiments were next carried out to see whether the biological activity could be preferentially extracted from the trichloracetic acid precipitates It was found that a 3 per cent solution of trichloracetic acid in 1N acetic

TABLE III

Fractionation of ACTH with TCA under various conditions

Starting Material	Conc TCA per cent	Total Activity Recovered	Activity ppt U/mg	Activity Extract U/mg
(1) Lyophilized ultra filtrate 2 2 U/mg 0 5 per cent Sol	15	75	8 8	—
(2) Trichloracetic acid ppt 4 7 U/mg	3 per cent in 1 N acetic acid	100	—	11
Trichloracetic acid ppt 4 7 U mg	5 per cent in 1 N acetic acid	80	—	6
(3) Lyophilized ultra filtrate 5 2 U/mg 0 5 per cent Sol	10	43	—	11
	15	80	—	21
	20	43	—	12

acid extracted all the activity with about half the weight so that the process gave an overall enrichment of 5–7 times The trichloracetic acid was removed from the extract by exchange for acetic acid using Amberlite IRA 400 acetate and the solution lyophile dried In this way preparations assaying up to 30–40 I U /mg can be obtained Such preparations are however extremely unstable and this property has seriously hindered their further investigation However it is possible that this difficulty may be overcome Fractions prepared in this way are of course grossly inhomogeneous and it is our opinion that they contain less than 10 per cent of the active principle The purification methods described here represent only a very small proportion of those tried in our laboratory Partition chromatography in a variety of systems counter current distribution adsorption chromatography on several adsorbents ion exchange separations on various cationic resins etc have

material was dissolved in 2N acetic acid and electrodialysed in a three compartment cell at a potential of 15–16 volts and currents of 200–250 ma The cathode liquid was changed continuously and collected for two 20 min periods 35 per cent of the activity was recovered in the first fraction and 60 per cent in the second The material in the first fraction had a potency of 6 I U /mg while that in the second had a potency of 15 I U /mg The original ultra-filtrate fraction had a potency of 7 I U /mg It thus appeared that the ultra-filtrate fraction contained material moving faster than ACTH as well as slower Similar results were found when using the Durrum paper iono phoresis method on ACTH fractions The electrodialysis method is thus capable of producing a 2–3 fold purification under favourable circumstances It is however very

TABLE II

Electrodialysis of ACTH

Starting Material	Time from Start Mins	Percentage Activity Recovered	U/mg
(1) Acetic ether ppt in 2 N acetic acid 1 0 U/mg	0–30 31–60	45 13	3 0 2 0
(2) Lyophilized ultra filtrate in 2 N acetic acid 2 2 U/mg	0–10 11–20 21–30 31–40	43 43 0 0	5 5 11 0
(3) Lyophilized ultra filtrate in 2 N acetic acid 3 0 U/mg	10–40	128	22 0
(4) Lyophilized ultra filtrate in 2 N acetic acid 4 0 U/mg	0–22 23–42	48 80	5 7 15 3

difficult to control all the variables concerned and studies on different types of membranes, multiple membrane cells, varying concentrations of medium etc , have not yet yielded a reliable method of operation Potencies of fractions obtained in this way have varied from 6–22 I U /mg It appears likely that the chief source of variation is the nature of the individual preparation under examination Some representative results are given in Table II

TRICHLORACETIC ACID FRACTIONATION

The behaviour of ultra filterable ACTH to a variety of acidic precipitation agents was examined These included picric picrolonic, helianthic, rufianic, flavianic rhodanilic, trichloracetic and several other acids With many of these the biological

correlated well with the therapeutic response This does not exclude the possible effects of other factors on potency as patients have intact pituitaries which might contribute to the response

We were interested in the other factors which may be present in crude pituitary extracts and which may inadvertently be administered to patients given crude ACTH *In the purification method illustrated on the slides* corticotropin together with a large proportion of the intermedin and adipokinin was concentrated by adsorption on oxycellulose and a partial separation of these three hormones effected by adsorption and distribution methods growth hormone was purified from the unadsorbed fraction

C H Li I agree with Dr Morris as to the importance of the properties of the membrane used in electrodialysis experiments

We have reported recently (Hess *et al J Am Chem Soc*) that electrodialysis of an acidic solution of the sheep hormone protein using a vegetable parchment in a field of high voltage gave rise to two main fractions of these, the cathode fraction, comprising from 15–20 per cent of the total material possessed almost all of the hormone activity In a typical experiment 40 mgm of the ACTH protein were dissolved in a 20 cc solution of pH 3 1 and put into the centre compartment the anode cell contained distilled water which was changed at hourly intervals during the 5 hour experiment *A current of 10–20 milliamperes* was *maintained by suitable variations* of the applied voltage (500–1500 volts) The results show that 21 per cent of the protein nitrogen migrated to the cathode and had most of the ACTH potency the starting protein had a specific activity of 2 3 U S P Units/mg whereas the potency of the cathode fraction was found to be that of 9 4 U S P Units/mg It should be pointed out that if the vegetable parchment membrane between the centre and the cathode compartments were replaced by goldbeater s skin no separation could be *obtained*

C J O R Morris Professor Li am I right in assuming that goldbeater s skin is a positively charged membrane?

C H Li It is

C J O R Morris A positively charged membrane is obviously best for separating cations but it may be difficult to get any cations through it at all

Professor Li did you say that you adjusted the pH of the centre cell with HCl?

C H Li Yes we continuously adjusted the pH in the centre cell by the addition of H Cl

C J O R Morris HCl being inorganic and having a small molecule it will be rapidly distributed to all three cells

C H Li I agree

C J O R Morris You are bound to get traces of salts in your extract and even small traces of salts can increase the weight of the extract by 5–10 per cent without of course any corresponding increase of active material

In all these ultra filtration techniques it is always necessary to pre dialyse the membrane before use preferably with 2 N acetic acid as even cellophane membranes contain traces of protein and copper

not yielded any superior separations The experiments described do however show that it is possible to obtain preparations of 30–40 I U /mg with very mild separation methods, so that it is extremely unlikely that prior hydrolysis of a parent molecule is necessary for the separation of ACTH of relatively low molecular weight This is certainly true of beef and pig ACTH and the few experiments we have carried out with sheep ACTH have not in our hands shown significant differences In fact any procedure involving hydrolysis has in our experience made subsequent purification more difficult

In conclusion we may perhaps add a word of caution It has become so usual to speak of ACTH peptides that it is often forgotten that the evidence for ACTH being of protein or peptide nature rests entirely on enzymatic inactivation experiments As these have been carried out on material which is still quite inhomogeneous, we believe that it is better to reserve judgment on the chemical nature of the hormone until much better preparations are available At present all that can be said is that ACTH appears to be strongly basic with a molecular weight under 10,000 but as mentioned in the beginning of this paper speculations as to its biological actions and chemical nature must await further progress in isolation

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Discussion

ON PAPERS BY (1) LI (2) DIXON STACK DUNNE AND YOUNG
(3) DEDMAN FARMER MORRIS AND MORRIS

Chairman E B Astwood

E B Astwood For several years we have been treating patients with preparations of corticotropin varying in potency by a factor of a hundred and made by different processes They were all assayed by the Sayers test and we found that this test

H Heller I notice, Dr Astwood, that on the second slide, you showed that intermedin and ACTH have been separated Would you confirm this and perhaps indicate what should be thought of Sulman s claim that the two substances are identical?

E B Astwood I agree with Dr Morris that it is possible to separate intermedin from ACTH

J A Lock We can separate intermedin from ACTH but in the early stages of purification the maximal activity of both occur in the same fraction

C J O R Morris With the Landgrebe method it is possible to separate intermedin completely from ACTH

W S C Copeman Has anyone any idea which fraction of ACTH carries the anti Rh and anti inflammation factors? Is it the ascorbic acid depletion factor? This would seem to be the conclusion to be drawn from this discussion, but there are strong clinical reasons for rejecting this view

C H Li With regard to Dr Vogt s point, that any injected protein can cause eosinopenia and that therefore this test is not reliable for indicating ACTH activity I would like to say that we consistently use serum albumin as a control protein and that we have also tried growth hormone as a control, but found that neither of these caused eosinopenia hence I think it is a valid test for ACTH activity

I was pleased to hear that Dr Lock used a high alcohol with his hydrolysate when using the charcoal method of separation as the higher alcohol prevents the ACTH from becoming too firmly attached to the charcoal The solvent used in our charcoal adsorption experiments was 50 per cent ethanol containing 0 1 M HCl

With regard to the actual form of ACTH the activity is certainly always associated with fractions which are peptide in nature and there is no evidence against its being a peptide I think the destruction of its activity by proteolytic enzymes supports the idea that it is a peptide

It may be that ACTH is present in the gland in a latent state perhaps bound to a protein as is thyroglobulin but I think that the active principle is a peptide

F G Young I agree with Dr Morris regarding the nature of ACTH and think the most important evidence is its destruction by proteinases I think we all agree that the active material contains a polypeptide

I think that the adrenal weight maintaining factor is different from the factor in pregnant mare s serum discussed by Reiss in his paper in 1940 I do not think this factor is specific for the suprarenals It certainly also works on the gonads but we have not yet tried it on other endocrine glands

With regard to Dr Overbeek s point—we have had very much wider ratios AAF/AWF 5 000/1 to 1/8 so I think that there must be two factors in ACTH It is perhaps worth emphasizing as a more general observation arising out of problems of this nature that these hormones have a half life in the circulation which is to be measured in minutes not hours Hence of course delayed absorption may in fact mean a greatly increased effective dose

I agree that the activity of acetone dried extract in this country is 0 2 units/mgm rather than 0 4 claimed by American workers Perhaps this is the fault of British pigs!

M Reiss We ourselves have claimed for several years the existence of a separate adrenocorticotrophic growth factor It is, therefore, very interesting that Professor Young apparently has isolated such a fraction from pituitary extracts However, during recent years we have become rather more sceptical about the existence of such a separate fraction This is due to our experience in the purification of other pituitary anterior lobe hormones When preparing thyrotrophic hormone it is found that with increasing purity of an extract, its ability to increase the weight of the thyroid diminishes The same holds good for gonadotrophic extracts prepared from the pituitary anterior lobe The purified extracts increase but little the weight of the ovaries Before accepting the existence of a special adrenocorticotrophic weight factor one ought to see whether such fractions do not influence the weight of thyroid and gonads in the same way as the weight of the adrenals The existence of a hormone produced in the pituitary anterior lobe acting as a growth factor for all the subordinate ductless glands alike cannot be excluded We shall show later, however, that the endogenous production of a separate ketosteroid and a separate corticosteroid mobilizing adrenocorticotrophic factor appears probable

The only conclusion one can draw from the different attempts at purification of the ascorbic acid reducing ACTH is that the hormone complex can exist in different molecular sizes It is probable either that a very small hormone complex can be adsorbed by protein complexes of different molecular size or that a simple prosthetic group is present on carriers of different molecular size The basic character of the purified extracts is particularly interesting It seems that when studying the chemistry of the hormone one will have to pay special attention to the basophil cells of the anterior lobe

The more recent conclusions of Li concerning the chemistry of ACTH cannot be accepted since the slope of his biological standardizations is very low, and the number of animals used per dose therefore too small

G A Overbeek With regard to the possible existence of two adrenotropic hormones, we made comparisons of the two types of activity (Sayers and maintenance tests) in a number of ACTH preparations obtained by a variety of methods and arrived at the following figures (AAF/AWF) 1 47, 2 23 1 66 0 65 1 42 1 23 1 20, 0 95 2 48 (Av = 1 5) These figures do not differ significantly from the average, and give no indication of the existence of two different factors

With preparations of ACTH suspended in beeswax mixtures and similar media, the activity as measured by the adrenal weight test was augmented more than ten fold This effect may be due either to delayed absorption or to inhibition of ACTH destruction

J A Lock We have tried concentration of ACTH by activated charcoal and found it very difficult with ox pituitaries We hydrolysed the extract with pepsin then tried the effect of adjusting the pH before using the charcoal technique and found that we got maximum protein yield at lower pHs maximum potency at alkaline pHs and combining these we found that we got the maximum total yield at a pH of 9

I should like to know whether Professor Li checks the pH in his charcoal technique

M Vogt May I give a word of warning about using the eosinopenic test for comparing protein and non protein material as many proteins alone will cause eosinopenia on injection?

The Suprarenal Cortex
The Structural Background

by

J M YOFFEY

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THE suprarenal cortex presents a number of interesting problems both of morphogenesis and histogenesis but perhaps the most intriguing problems of all are those which arise out of a consideration of the relationship between its structure and its function In this communication I should like to indicate briefly the lines along which our own views have been tending to develop

The earliest studies of the suprarenal cortex were chiefly morphological It was Arnold (1886) who first gave us the subdivision of the cortex on purely morphological grounds into the traditional three zones glomerulosa fasciculata, and reticularis These three zones are to be found in most mammals though occasionally one of them may be disproportionately well marked or the reverse Thus Bourne (1934) has shown that the Australian opposum has no glomerulosa a zone which is also poorly developed in the lemur and monkey (Kolmer 1918) whereas the bat possesses little or no reticular zone If one takes two common laboratory animals the rabbit and the guinea pig the former possesses a relatively narrow reticularis and wide fasciculata while in the latter the reticularis is very well marked and usually exceeds the fasciculata in width

Once the three zones had been defined an obvious question to arise was that of the relation between them and also whether they were absolutely distinct from one another or not At this stage Gottschau (1883) introduced the concept of the inward migration of cells from the outer part of the cortex the cells traversing in succession the various cortical zones and finally ending in the medulla where they underwent degeneration Gottschau did not distinguish clearly between the inner part of the cortex and the medulla but regarded them as a single zone which he termed the *zona consumptiva*

When it became established that cortex and medulla were fundamentally different Gottschau s hypothesis was obviously not acceptable in its entirety However ideas once current are notoriously difficult to dislodge and Gottschau s view persisted in a modified form according to which cells were believed to be continually migrating from the periphery of the gland to the reticularis With this went the inference that the oldest cortical cells were those nearest the medulla and these were therefore regarded as senescent or degenerating This concept was substantiated by the findings of later workers e g Graham (1916) Salmon and Zwemer (1941) Salmon and

of cell death, it is of interest that quite a number of dead cells of this nature were found in the *zona fasciculata* Bennett concluded however that 'these should not be regarded as having become senescent in the ordinary way but as having died prematurely Further Bennett could find no evidence to suggest that the dying cells underwent phagocytosis and the precise mode of their disappearance—if they were present—remained something of a mystery

Hoerr (1931) noted that in the guinea pig the cells of the reticularis fell into two main categories light and dark, of which the light cells were often the largest cells in the gland larger than what he considered to be the actively secreting cells of the *zona fasciculata* In discussing the significance of these two cell types he referred to an earlier observation of Cowdry (1922) to the effect that the large light and the smaller dark cells were different functional stages of the one cell type which presumably could only mean that the cells of the reticularis were constantly discharging and refilling

With the discovery of the influence of the anterior lobe of the pituitary upon the suprarenal cortex and the subsequent isolation of ACTH significant new observations were made on the activity of different cortical zones It soon became clear that the glomerulosa was far less dependent upon the hypophysis than were the other two zones The glomerulosa seems to function quite well and appears to be more or less normal whether the hypophysis is present or not but the *zona reticularis* and *fasciculata* undergo atrophy following hypophysectomy It is significant however that the first obvious change after hypophysectomy is in the reticularis and degeneration in the fasciculata only occurs subsequently to this

Thus Houssay and Sammartino (1933) found that in hypophysectomized dogs the first zone to show degeneration was the reticularis the fasciculata showed similar changes a little later though even here the inner portion of the fasciculata degenerated before the outer L atrophie corticale est la consequence de l atrophie de la reticulee puis de la partie interne de la fasciculee et en fin de toute celle ci Par contre la couche glomerulaire se conserve et dans la plupart des cas s hypertrophie surtout quand les couches internes de l ecorce sont tres atrophiees The hypertrophy of the glomerulosa may be more apparent than real but the earlier onset of changes in the reticularis seems perfectly definite and has been substantiated by other workers e g Crooke and Gilmour (1938) Shumacker and Firor (1934) The latter observers hypophysectomized rats and examined the adrenals at varying periods after operation At five days there was some distortion of the reticular zone and the cells in this layer and in the inner part of the fascicular zone were smaller than normal At eight days the reticular zone was hardly recognizable, and the fasciculata showed more marked changes

The results of hypophysectomy then seem to indicate that the *zona reticularis* is the most sensitive of all cortical zones to lack of pituitary stimulation There is also more positive evidence pointing in this direction Thus Bergner and Deane (1948) found in rats that injections of ACTH for twelve days produced hypertrophy of the reticularis Sonenberg *et al* (1951) using ACTH labelled with I^{131} found that it appeared to be localized mainly in the reticulari Nichols and Little (1951) investigated the effect of ACTH upon the oxygen consumption of the various zones of the adrenal cortex They found that in the resting state slices of the outer part of

Zwemer used vital dyes to label the subcapsular cells, and they claimed that by this technique they could demonstrate the inward migration of these cells beyond question They even drew up a timetable for the process, and estimated that in the rat 20–30 days were required for the complete migration of cells from the capsule to the reticularis At a later date Greep and Deane (1949) studied the regeneration of the cortex after enucleation of the gland Here there appears to be no doubt that the cortex regenerates by the inward migration of cells from the subcapsular region though apparently the new cells are formed not from the capsule itself but from immediately adjacent cells which are left behind at the time of enucleation

However, there is an impressive array of facts against the concept that the reticularis is a zone of degeneration The vital dye experiments just quoted were repeated by Calmo and Foster (1943) and in our own laboratory by Baxter (1946), neither of whom was able to confirm Salmon and Zwemer s findings It is worth noting incidentally that it has been somewhat unfortunate that so much attention has been devoted to the rat It is only in a small adrenal that the hypothesis of the inward migration of cells becomes really plausible since there would be a relatively short distance for the cells to traverse whereas in larger adrenals the distance to be covered by the migrating cells is considerably greater, even though apparently the thickness of cortex does not increase indefinitely with the size of the animal Furthermore the glomerulosa in the rat is relatively poorly developed If one looks at the adrenal of an animal such as the horse, not only are the cells of the well developed glomerulosa strikingly different from those of the underlying fasciculata, but there is an abrupt transition from one to the other, with no suggestion of any intermediate forms between the cells of the two zones

Mitoses can occur in the reticularis though admittedly they are not as frequent as in the other zones For example Hoerr (1931) in a paper which is generally adduced in support of the view that the reticularis is a degenerative zone, found in the guinea pig that during recovery from chloroform poisoning the reticularis contained on occasion an appreciable number of mitoses In the rat on the other hand, Mitchell (1948) was unable to observe mitoses in the reticularis In the mouse, Jones (1950) found that the *zona reticularis* was well developed in the adrenal of the normal parous female but either very poorly developed or even absent in the male In certain cancerous strains the reticularis in the female showed well marked brown degeneration Though on the whole Jones favoured the theory of inward migration there were several awkward facts which he found difficult to fit in with this theory and one is left with the feeling that the marked increase of degeneration in the cancerous strains may well be the reaction of the inner zone to unknown toxic factors

This brings us to one of the difficulties in accepting the view that the reticularis is a zone of degeneration, namely the fact that so very often degenerating cells may be singularly difficult to detect in the reticularis of the normal suprarenal except occasionally for a very few close to the medulla The word normal needs to be stressed in this connection for the reticularis is the most sensitive part of the cortex and is most readily damaged by harmful stimuli Whitehead (1943), in guinea pigs up to six months of age could find no sign whatever of cell degeneration in the reticularis Bennett (1940) using the intravitam trypan blue technique thought that he could in this way detect dying cells in the reticularis Apart from the validity of his criteria

of the best examples is that reported by Zaleski (1934) in the thirteen lined ground squirrel, more especially in the female during pregnancy In these animals cortical hypertrophy is associated with expansion of the *zona reticularis* (cf Bennett s observa tions in the cat), 'and the differentiation within it of a characteristic highly developed outer sub zone termed reticularis A

Whitehead (1942) in the fasting guinea pig found the *zona intermedia*—though he did not name it as such—very distinctly brought out after nine days of starvation by its lipoid depletion, giving rise to a lipoid free zone between the fasciculata and the inner part of the reticularis The so called siderophilic zone (vide Bennett 1940) is in the same intermediate position The deep zone of the fasciculata described by Goormaghtigh (1922) seems to include adjacent strips of fasciculata and ret icularis

More recently Seligman and Ashbel (1951—and earlier papers) have used 3 hydroxy 2 naphthoic acid hydrazide for the histochemical demonstration of carbonyl groups and give reasons for suspecting that this material might possibly be a keto steroid However their results were only obtained in formalin fixed material formalin fixation appears to have singular effects upon the cells of the adrenal cortex (Yoffey and Baxter 1947, Feldman 1950) On the whole many workers have felt inclined to agree with Sarason (1943) that a straightforward lipoid stain appeared to give as much information as any of the histochemical tests

Our own group, after numerous trials finally decided to concentrate on the Schultz reaction for cholesterol which appeared to be generally accepted as the precursor of the steroid hormones Cholesterol possessed the great advantage of being present in the gland in appreciable amounts, unlike the steroid hormones which were to be found in minute quantities Furthermore the Schultz reaction purported to be an application to sections of the Liebermann Burchard test generally used by chemists for the quantitative estimation of cholesterol its chemical specificity appeared there fore to rest on a secure foundation Variations in the cholesterol content of the gland as measured by the intensity of the Schultz reaction might then be interpreted as throwing light on the conversion of cholesterol into the actual hormones

In our earlier work (Yoffey and Baxter 1949 Robinson and Yoffey, 1950) we obtained results which appeared to be highly significant It was found for example that after the injection of cortical extract for several days the Schultz reaction appeared to be intensified This was interpreted to mean that in the absence of need for endogenous ACTH and steroid hormone production cholesterol as the precursor of the hormones accumulated in the cortex as such To this phenomenon we applied the term loading and not infrequently a similar loading was observed in rats isolated for seven days In this latter case the loading would presumably be due to the absence of the normal stress reactions which are no doubt inevitable when several rats are housed together in a single cage

Judging by the Schultz reaction the reticularis certainly contains much less cholesterol than the fasciculata though it usually contains some with the exception very frequently of a narrow juxtamedullary zone which may on occasion be com pletely Schultz negative Stress stimuli such as cold or the injection of adrenaline (Robinson and Yoffey 1950) seemed to produce quite rapid depletion of cholesterol from the reticularis which appeared to empty before the fasciculata and in which

the cortex, i e consisting of capsule, *zona glomerulosa*, and a strip of fasciculata had a higher rate of oxygen consumption than did slices of *zona fasciculata* and reticularis However, the addition of ACTH increased the oxygen consumption of both slices to an approximately equal extent Their figures for the oxygen consumption of the outer slice are close to those of Tepperman (1950) for slices of the whole cortex

Another approach to the problem has been by means of histochemical techniques but these on the whole have been singularly disappointing The ideal procedure is obviously that which would demonstrate the existence and distribution of the actual steroid compounds, and the pioneer attempt to achieve this we owe to Bennett (1940), who thought that phenylhydrazine could be used as a specific reagent for the staining of keto steroids He concluded on the basis of his phenylhydrazine studies that the cortex could be divided into four zones from without inwards, namely pre secretory secretory, post secretory, and senescent Compared with Arnold s subdivision the pre secretory zone corresponded approximately to the glomerulosa the secretory to the outer fasciculata, the post secretory to the inner fasciculata and the senescent to the reticularis

However although Bennett described a senescent zone, he was not altogether happy about it 'When hypertrophy' [of the cortex] takes place with maturation in male cats or with pregnancy the widening of the cortex does not affect the secre tory zone but on the contrary involves the cells of the innermost zones i e his senescent area On the basis of the phenylhydrazine reaction these cells could not possibly be making corticosterones but he thought that they may be engaged in producing other substances or hormones not yet identified

In agreement with several other investigators, we ourselves (Yoffey and Baxter, 1949) were unable to accept the specificity of the phenylhydrazine reaction and found that with occasional exceptions whose significance we were unable to explain the phenylhydrazine, the plasmal and the Schultz reactions corresponded on the whole fairly closely with one another and also with the ordinary Sudan stains Using these as non specific stains it could nevertheless be shown that depletion and accu mulation of stainable matter were typical of different phases of gland activity It was further noted that under the influence of stimuli such as ACTH known to produce rapid output of steroids, it was always the reticularis which showed the first signs of depletion of stainable matter On the basis of these observations it was suggested that the reticularis was certainly an actively functioning zone possibly one in which hormones were stored filling up again after its depletion with products brought to it from the fasciculata

This would make changes in the fasciculata secondary to those in the reticularis If this hypothesis be correct the junction between fasciculata and reticularis should be on occasion a zone of special activity We did in fact not infrequently find evidence which seemed to indicate a depleted reticularis just beginning to fill up (Yoffey and Baxter, 1949, p 1 Figs 9 and 10) and giving rise to a temporary intermediate zone between the fasciculata and reticularis

When one surveys the literature it is interesting to find how frequently an inter mediate zone does in fact crop up under a variety of names, between the fasciculata and the reticularis From its position such a zone has been regarded as either a specialized outer portion of the reticularis or an inner zone of the fasciculata One

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we frequently thought we could detect signs of cholesterol depletion in as little as five minutes after the exposure to cold or the injection of adrenaline

If the Schultz reaction really were so sensitive an indicator of cortical changes, it would obviously have a wide range of application It therefore appeared well worth attempting to correlate the histological appearance with an actual chemical estimation of cholesterol or cholesterol esters This was done in eighty rats (Ashford Robinson and Yoffey, 1950—unpublished) with the result that it was not found possible to establish a constant relation between the intensity of the Schultz reaction and the cholesterol content of the gland

Apart from the secretion of any other steroid hormones, the reticularis has been considered by a number of workers to be responsible for the production of androgens An excellent review of the earlier work is given by Parkes (1945) Whatever may be the position in the animal adrenal there seems to be no doubt that in a number of cases of virilism in man there is undoubted hypertrophy of the *zona reticularis* A paper such as that of Blackman (1946) seems to establish a relationship between the reticularis and different types of virilism very convincingly It is of especial interest that in some of these cases the *zona reticularis* forms the major part of the cortex and under such circumstances its origin from a thin outer shell of cortical tissue seems especially difficult to accept

On the whole then, we feel that the *zona reticularis* may well be a morphologically independent zone, that it is a zone which is actively functioning, and that it is the first part of the cortex to react to stress stimuli it does not consist essentially of dead and dying cells

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The Nature of the Adrenal Cortical Secretion

by

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I PROPOSE to discuss our problem under four main headings

(1) What hormones are produced by the adrenal cortex?
(2) How do they act in metabolism?
(3) On what part of the cell do they act?
(4) When are they produced?

(1) WHAT HORMONES ARE PRODUCED BY THE ADRENAL CORTEX?

This first question is easier to answer to day than it was even a year ago It has been known for long that of the twenty nine or more corticosteroids identified in cortical extracts only about six are active Desoxycorticosterone (DCA) is present in the cortex only in small amounts but the synthetic substance has been freely available for some time, and differences in action between it and the cortical extracts were early noted DCA is still the substance which keeps adrenalectomized animals alive in the smallest quantities The properties of cortisone, on the other hand were not appreciated for some time and in fact Kendall in 1934 regarded it as unable to maintain life in adrenalectomized animals while it even decreased the body weight of normal animals

Advances in analytical methods mainly paper chromatography have now made possible a direct attack on the problem Bush (1951) has shown that the blood contains only corticosterone and 17 hydroxy corticosterone The proportions of these substances vary in different species ranging from 1 10 in the rabbit, to 10 1 in the monkey Stimulation of the adrenals by ACTH does not alter these proportions (Pincus 1951)

In the urine of man only 11 oxycorticoids have been found by most investigators. Like others we found with paper chromatography in the urine of man about 50γ/day while when using formaldehyde or other reduction methods up to 1000 γ/day are assayed certainly different substances I know of only one group (Staudinger and Weissbecker) who claim to differentiate between DCA and 11 oxycorticoids in the urine

Pregnanediol is the excretion product of DCA as also of progesterone It is present in the urine of females, and not of males, but it appears after the injection of DCA in about 10–20 per cent of the quantity injected This also makes it improbable that DCA is excreted as a normally occurring hormone

Thus the actual determination of corticoids in blood and urine is clearly not in accord with the view that 11 desoxy corticoids the so called mineralo corticoids, are

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Discussion

Chairman H Heller

S *Zuckerman* Professor Yoffey, can you tell us anything about the x zone and its relation to the inner part of cortex?

J M Yoffey We have concentrated on the adult cortex and have not as yet investigated the developing gland so I am unable to answer your question

The difference between foetal and adult cortex, as suggested by Blackman in 1946 is probably not so great as is generally thought

Celestino da Costa I am impressed by the importance of the intermediate zone

I think that the different response of the different zones is due to local conditions in the gland

The different zones vary markedly from animal to animal e g the glomerulosa is absent in bats, and hence I think that it is dangerous to generalize about them

products of steroid synthesis or breakdown in the adrenal cortex? It is clear that the 'sexoids'—as they have latterly been termed—of the adrenal cortex cannot have an important physiological role, for the symptoms of castration always supervene after gonadectomy. If on the other hand cats are adrenalectomized and kept alive for years with DCA, their sexual behaviour and powers of reproduction are unchanged as we showed ten years ago. Hence we conclude that androgen production is not a physiological activity of the adrenals but might become a pathological one.

Histological studies have often encouraged the idea that there are several adrenal hormones. There is no doubt that the cortex of most mammals possesses three distinct layers, *zona glomerulosa*, *fasciculata* and *reticularis*. This appears to fit in well with the triple hormone theory. But histologists have repeatedly suggested that this may be an accidental differentiation, probably caused by the growth of the cells from various directions (Celestino da Costa, 1950, 1951).

If we look a little further afield at the adrenal cortex of birds, reptiles and amphibia we see no such differentiation of the cortex into three layers, but nevertheless the production of cortical hormones seems to be the same as in mammals.

After Grollman (1936) first suggested that the x zone of the mouse's adrenal produced sex hormones, the same thing was held to be true for the *zona reticularis*, which is however a different structure. The x zone is foetal adrenal tissue which disappears in different animals at various stages of development. The presence of 17-ketosteroids in the *zona reticularis* could be interpreted as proof of sex hormone production, but even if undoubtedly present they could be regarded as intermediary corticoids, as already mentioned.

Similar complicated proofs have been brought to show that the *zona glomerulosa* produces the mineralo-corticoids or electrolyte hormones. Greep and Deane (1949) came to this conclusion because in the hypophysectomized rat it is only the *zona fasciculata* and *reticularis* which undergo atrophy, while at the same time the Na and K content of the blood are normal. Hence the *zona glomerulosa* was regarded as the site of production of mineralo-corticoids, while the two other zones were held responsible for the production of carbohydrate corticoids.

But in the hypophysectomized animals all metabolic processes are diminished, while ACTH increases them all. Lewis (1942), Ingle (1947), and Levin (1949) showed that when ACTH is produced in response to stress, the metabolic changes involve all spheres of metabolism (Bush, 1951).

(2) HOW DO CORTICAL HORMONES ACT IN METABOLISM?

We are thus confronted with the question:

How do the adrenal cortical hormones act on metabolism if there are at least two different hormones or groups of hormones, namely mineralo- and gluco-corticoids? Or alternatively if there be only one hormone how can it influence so many varied processes?

From a historical point of view it was precisely this multiplicity of activities which afforded the main reason for supposing that several hormones exist. Increase of urea in the blood plasma was taken to mean a disturbance of protein metabolism or kidney function. Increase of glycogen was explained by the action of carbohydrate

normally present in the blood though they might conceivably be so under pathological conditions As far as I know however, this has not yet been demonstrated

Selye (1951) holds a different view, and in the *Symposium on Steroids* (p 18) states Since there can be no doubt about the normal occurrence of desoxycortisone (or 17 hydroxy 11 desoxycorticosterone Reichstein s Compound S) in the adrenals we may safely conclude that natural mineralo corticoids do possess toxic effects upon the kidney and the cardiovascular system *it appears highly probable that* desoxycortisone and desoxycorticosterone like "mineralo corticoid compounds,'play an important role in physiology and pathology ' Dobriner (ibid p 23) agrees that Compound S is an important adrenal hormone I believe that Compound S (constitutes) a *significant fraction of the steroids secreted by the adrenal* (See however the new work of Tait Simpson, and Grundy, 1952)

Fresh light has been thrown on the problem by experiments on the fate of DCA in the body Pincus and his co workers (1949–50) showed that DCA, perfused *through isolated adrenals* is *oxidized to* 11 oxycorticoids Dorfman s group (1949) showed that slices of adrenal cortex *in vitro* can oxidize DCA to 11 oxycorticoids, which are recognized by their 'carbohydrate activity Subsequently Kahnt and Wettstein (1951) demonstrated the same change with liver kidney and other tissues, desoxycorticosterone becomes corticosterone, Compound S becomes 17 hydroxy corticosterone, and it has been claimed (Seneca *et al*, 1951) that homogenizates of liver kidney, testis and ovary will even produce cortisone It is clear then that injected desoxycorticosterone is oxidized in a number of tissues to 11 oxycorticoids This is in accordance with the view which we ourselves have put forward for many years, that there is no qualitative difference between the action of desoxy and other corticosterones but that the desoxy compounds merely act more slowly than the other substances into which they are ultimately transformed

The findings of the past few years make it much easier now to discuss the question of the multiplicity of cortical hormones The manifold changes occurring in the body after adrenalectomy gave rise at first to the view that they could not all be due to lack of a single substance This view was strengthened by the observation that there were differences in action between various cortical fractions, in addition to the difference already noted between DCA and 11 oxy compounds Thus there were recognized a kidney factor (Kendall) a Na factor (Hartmann) a carbohydrate factor and a life maintaining factor in the amorphous residue There were E S and N hormones while DCA became the prototype of the mineralo corticoids *since it* was inactive in a six hour test on glycogen production whereas corticosterone or cortisone were active, and thus became glycocorticoids But since we now know that only 11 oxycorticoids are present in the blood and that 11 desoxy compounds are only intermediate cortical products which are *finally oxidized to 11 oxy com*pounds the so called mineralo corticoids can no longer be reckoned as true cortical hormones

A third group of cortical compounds to be considered are the sex hormones There is no doubt that *adrenal cortical tumours or hyperplastic* adrenals may produce large amounts of androgens, and furthermore that the normal adrenal contains large quantities of 17 ketosteroids, some of which may be androgenic Are they the N hormones of Albright with protein anabolic activity? Or are they merely intermediate

for direct use in energy production If there is enough glycogen present in the muscle, then this is used, if glucose is needed, however, and glycogen is not present, then proteins are used for the production of glucose or even fatty acids The main action of the corticoids then appears to be to prepare glucose for the liberation of energy surplus glucose being stored as glycogen or fat The corticoids therefore may be envisaged as possessing a 'switchboard function, leading either to a glycogen → glucose or protein → glucose transformation on the one hand or glucose → glycogen, glucose → fat on the other, according to the availability of glucose or the need for it

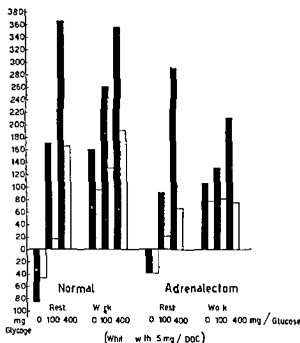


Figure 1 Glycogen metabolism of diaphragm of rat Each column represents the middle value of a series of experiments (for details see the paper of Mentha Vogth and Verzar 1948) *Black columns without white columns with the addition of desoxy corticosterone Each series contains experiments without with* 100 *and with* 400 *mg per cent glucose Work experiments are done with direct rhythmical stimulation Notice all values with adrenalectomized animals are smaller than with normals*

I believe therefore that all the energy production of the cell catabolic as well as anabolic is connected with the activity of this hormone

All this however is only one side of the action of cortin It has been known for about twenty five years that adrenalectomy is followed by major disturbances of Na and K as well as water metabolism Na decreases in the blood plasma K increases and these changes are reflected in the urinary excretion of these minerals Water and sodium are partly moving into the cells while potassium is leaving muscle fibres and probably other tissues also

factors Na decrease in the blood plasma, or the adverse behaviour of K after adrenal ectomy, was attributed to lack of an electrolyte factor It was in 1941, when we kept adrenalectomized cats alive with DCA for over a year, that we first began to doubt this qualitative differentiation, allowing of course for quantitative differences in the velocity of the reaction Yet if only one hormone is produced, how can one explain so many diverse effects? I think adrenal cortical research has now led to a deeper understanding of, if not the essential unity, at any rate the intimate connection be tween metabolic processes of such widely different types

There is no doubt about the disturbance of glycogen production in the adrenal ectomized animal It is completely corrected by adreno cortical extracts, rapidly by cortisone and rather slowly by DCA Adrenalectomized animals kept alive by DCA have normal glycogen content This was shown by us, in 1941 (Verzar *et al*) and again in 1949 (Sass Kortsak, Wang and Verzar 1949) Segaloff (1951) has recently shown (as had Derman with us in 1946) that the phlorizin diabetic animal after desoxycorticosterone excretes increased quantities of glucose But it is also true that in the six hour test of Reineke and Kendall (1943) no activity of DCA can be seen

Long (1949) and his school thought that 11 oxycorticoids induced protein break down with the formation of glucose in the starving animal since together with glu cose N also is excreted in the diabetic rat But it was found by Engel *et al* (1949) that no protein will be broken down if amino acids or glucose are given together with cortisone Similarly in an experiment with Wang (1949) we showed that glycogen production from glucose given by stomach tube, was also increased by cortisone The action of 11 oxycorticoids is thus either directly on glucose or on glycogen pro duction, but not necessarily on proteins It has long been known that even fatty acids can be used as glycogen producers (Thaddea 1938, Stetten, 1945–46)

It was with some embarrassment therefore that we found that the isolated mam malian muscle of normal or adrenalectomized animals did not produce glycogen, but that a distinct inhibition was seen in every phase of glycogen metabolism (Mentha, Vogtli and Verzár 1948) (Fig 1) This inhibition might be explained as a non specific steroid effect, or as a competitive inhibition, or else one might search for parallels in other observations such as the obvious antagonism between insulin and cortin in normal animals The former increases glycogen production in muscle the *latter decreases it In the intact animal insulin hypoglycaemia is* counteracted by corticoids Minced muscle or liver tissue *in vitro* break down glycogen by phosphory lase activity This glycogenolysis is diminished in the adrenalectomized animal and can be restored with corticoids (see Verzar 1951)

Ingle (1949) working with eviscerated rats concludes The data emphasize the inhibitory effect of cortical hormones upon carbohydrate utilization rather than its positive effect upon gluconeogenesis 'Adrenal steroid diabetes is an overdosage phenomenon and 'there is some evidence that the cortical hormones favour the storage of fat

We have therefore the impression that the inhibition of glycogen production by corticoids is not an artefact, but has to be worked into the picture of their influence on carbohydrate metabolism *Corticoids do not directly increase glycogen production* or protein breakdown but they further gluconeogenesis the formation of glucose

cell and more Na^+ outside There is an exchange with Na^+ ions if K^+ leaves the cells and with Hodgkin (1947) one may speak of a Na pump which drives Na out of the cell during recovery after it had temporarily replaced K The contrary is believed by Shanes (1951) who emphasizes that the primary change is the escape of K which occurs even when Na cannot enter because of its low concentration out side the cell or fibre The restoration of polarization, the reabsorption of K depends *on carbohydrate metabolism If glucose is added, K re enters and Na leaves the cell* but if carbohydrate metabolism is inhibited by poisons such as iodo acetic acid

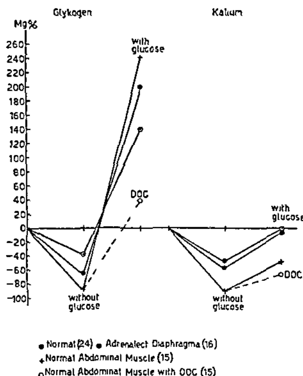


Figure 2 Glycogen and potassium changes in the surviving diaphragm of the rat and the abdominal muscle of the mouse (After Verzar and Leupin 1951)

this change does not occur We have discussed elsewhere (Verzar—Muskelkontraktions theorie 1943) the decisive role of potassium in the excitatory process in muscle (See also Fleckenstein 1942 1950)

Returning to the adrenal cortex it seems obvious that the *changes in K and carbo* hydrate metabolism are basically connected with one another and should not be considered as isolated phenomena It seems probable that the basic disturbance in the metabolism of glucose so essential for energy production is also responsible for the changes in potassium and in addition probably for the altered metabolism of Na and water

It was primarily the direct analyses of blood which convinced us that it does not

Now it is certainly a mistake to regard these changes as general disturbances of mineral or electrolyte metabolism, because Na and K have such completely different roles in metabolism while Na is present mainly in the body fluids and only in minute amounts in the cells, the contrary is true for potassium Changes in the Na content of the blood plasma occur mainly for osmo regulatory purposes Thus if water passes from the blood plasma into the tissue cells, Na is immediately excreted by the kidneys

One can consider the changes of water and electrolyte metabolism after adrenalectomy either from the point of view of the tissues—as we do—or as a result of a primary change of the activity of the kidney, possibly under the influence of the anti diuretic hormone of the posterior pituitary—as Gaunt (1949, 1951) has done There is no doubt that osmotic changes in the blood might stimulate the secretion of the antidiuretic hormone which might then lead to the decrease in water excretion by the kidney followed by an increase in excretion of the surplus NaCl

However, we think that the primary changes after adrenalectomy are in the tissues (extrarenal factors of Gaunt) *These are metabolic changes which lead to an uptake* of water and a release of potassium by the body cells This is the site of action of the hormone

This water movement after adrenalectomy leads to the haemo concentration which is the most decisive factor in the acute crisis of the adrenalectomized animal causing death through circulatory failure The haemo concentration can be counter acted by giving additional NaCl However the excellent results of such therapy have led to the erroneous belief that NaCl can keep adrenalectomized animals alive and in good health indefinitely Lewis *et al* (1942) Richter (1948) Ingle (1950) and Sayers (1950) have all pointed out that these animals are not normal and cannot withstand stress To this we would add that their growth is normal only for a short time, not longer than two months after which it declines and the animals die showing the same metabolic disturbances as the untreated adrenalectomized animals But about 30 per cent —in our rat colony—survive and after some time grow even more rapidly than at the beginning of the experimental period, finally they survive even without extra NaCl The NaCl therapy has allowed time for the regeneration of accessory adrenal tissue McFarland (1945) and Gaunt (1949) made similar observa tions We had the same kind of result in adrenalectomized rats maintained with DCA which could be discontinued after a while without detriment to the animals

The role of K in the body is completely different It is intracellular, but leaves the cell during periods of activity, as is well known in the case of muscle and nerve It should perhaps not be forgotten that it was largely through the adrenal cortex that attention was drawn to the relation between carbohydrate metabolism and the move ment of potassium (1943) Potassium moves into isolated yeast cells leucocytes (Palver and Verzar 1940) or muscle fibres (Leupin and Verzar 1950) if glucose is metabolized to glycogen but leaves the cells again if glycogen is broken down (Fig 2) Conway (1945) and Roberts (1949) have thrown light on this mechanism the latter describing a fructose—dipotassium phosphate during fermentation

This reaction is of especial interest to day, when polarization of the cell membrane is regarded as the basis of excitability or energy production in nerve and muscle fibre and probably other cells Polarization means that there are more K^+ ions inside the

The formation of glucose from proteins lends equal importance to the work of Kochakian (1950), who showed that arginase activity in the liver decreased after adrenalectomy He doubted the connection because of the time relations but this need not be an insuperable objection The inhibition by corticoids of glucose oxida tion (Gordan and Elliott 1947), and of D amino oxydases (Dorfman *et al*, 1950) points also to activities related to carbohydrate metabolism and is especially striking in view of the inhibition of glycogen metabolism by corticoids in the whole muscle Oxydo reductions may play a part in the rebuilding of the phosphorus donor ATP but little is known about its connection with corticoids

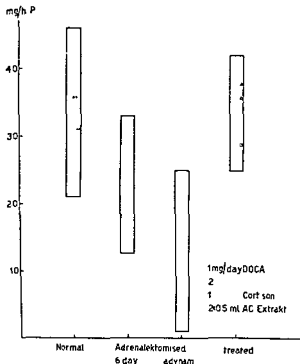


Figure 3 Alkaline phosphatase of the duodenum and jejunum of the rat (After Verzar and Sailer 1952)

It is perhaps too soon as yet to be dogmatic about the enzymological side of the problem but it is our belief that the further study of enzymes may well lead to an understanding of the basic reaction which is influenced by corticoids In 1939 I said We see in cortin a hormone influencing cellular metabolism in a basic way It is connected with oxido reductive phosphorylations Metabolic processes depending on these are disturbed To day we might add The phosphorylating enzymes of carbo hydrate metabolism are also responsible for the polarization of the cell membrane and thus for energy production It is here that the corticoids seem to act and here too is to be found the link between the metabolism of carbohydrate/protein on the one hand and of electrolytes (K/Na) on the other Sayers (1950) is also of the opinion that

contain two distinct types of steroid hormone namely mineralo and gluco corticoids Clearly the changes in Na, K, H O, carbohydrate, and other metabolites must be due to qualitatively similar hormones Hence it has been particularly important to be able to demonstrate (*a*) that the prototype of the so called mineralo corticoids, desoxycorticosterone, has a distinct action on carbohydrate metabolism both in the intact animal and on isolated tissues, (*b*) that the 11 oxy steroid cortisone, the best known gluco corticoid, restores Na and K to normal in the adrenalectomized animal (Wirz 1950–51)

Only three years ago it was anathema to believe that all active corticoids have qualitatively similar actions, but I am glad that this view is now also held by Ingle (1949) who said 'There is considerable overlapping of effects and there is a good deal of evidence that there are basic relationships between the distribution of in organic ions and the metabolism of organic compounds which relate to adrenal cortical functions Even Selye (*Symposium on steroids* 1951, p 25) admitted I certainly agree that there is no such thing as a *pure* glucocorticoid compound

(3) ON WHAT PART OF THE CELL DO THE CORTICAL HORMONES ACT?

Analysis of all the evidence leads us therefore to the conclusion that under physio logical circumstances only 11 oxy corticoids are produced, and that the various meta bolic changes influenced by withdrawal (adrenalectomy) or administration of corti coids are inter connected through a basic cellular process on which the hormone acts Our next question then is to discover what this process is

An analysis of carbohydrate metabolism has led to the conviction that corticoids act on some basic cellular enzyme process This concept originated in experiments on the active passage of hexoses through a single epithelial layer, the selective absorp tion of glucose and galactose in the intestine Since this was inhibited by enzyme inhibitors such as iodo acetic acid, and by phloridzin (Lundsgaard 1933, Bruckner 1951) and since we found the same decrease of selective absorption in adrenalecto mized animals even when kept alive with NaCl (Verzar and Sailer, 1952) (though there was some disagreement about this) it was thought that phosphatases might be the enzymes concerned

The alkaline phosphatase content of the intestinal mucosa (and of the absorbing epithelium of the kidney tubules), is considerably decreased after adrenalectomy (Kutscher and Wust, 1942), and restored by corticoids (Verzar Sailer and Richterich 1952) (Fig 3) This is clearly shown in the histological demonstration of alkaline phosphatase by the Gomori method as used by Verne (1948) for the intestinal mucosa and in our own laboratory for the intestine (1952) (Fig 4) and for the renal epithe lium (Tissieres 1948) Both the depletion and reaccumulation of alkaline phospha tase in the intestine was demonstrated It has further been shown by us both in muscle and liver that phosphorylase activity is decreased after adrenalectomy (See my summary of 1951)

From the work of Conway (1946) it would appear that the activity of muscle glucomutase is diminished so that the proportion of glucose 1 phosphate to glucose 6 phosphate changes from 1 2 to 2 1 and Cori s group (Sutherland *et al* 1941) has shown that hexokinase is antagonized by corticoids *in vitro*

Vogt (1943) showed that adrenaline stimulated the production of corticoids, and thought that it acted directly upon the cortex whereas Long (1949) believed that adrenaline acted by stimulating the anterior pituitary to release ACTH Sayers (1950) pointed out that a number of stimuli might first lead via the sympathetic to the production of adrenaline, which in turn would increase the output of corticoids either directly or by means of ACTH It is also possible that stimuli reach the anterior pituitary from hypothalamic centres either through nervous pathways or by means of a portal system Pituitary transplants to the anterior chamber of the eye may well provide the final answer

Whether or not the adrenal cortex can be directly stimulated in the various ways mentioned there can be no doubt that the most powerful stimulus is ACTH to which the cortex reacts very rapidly by the release of corticoids, as well as with hypertrophy if there is continued stimulation with ACTH What then is responsible for the secretion of ACTH? Is it only adrenaline? One is reminded of the discussions on the role of adrenaline in Cannon's work (1914) on psychic stress ('major emotions') There also the question arose whether the physiological concentration of adrenaline in the blood was high enough to give rise to continuous stimulation

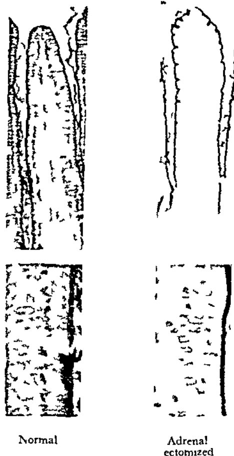
The main stimulus for the release of corticoids seems to be the concentration of corticoids in the blood it is still not perfectly clear whether this acts only via the anterior pituitary or also on the adrenal cortex directly Lowering of the blood corticoids after unilateral adrenalectomy leads to hypertrophy of the remaining adrenal If an adrenalectomized animal is kept alive with small doses of DCA accessory adrenal bodies will hypertrophy On the contrary large doses of cortisone lead to an atrophy of the adrenal cortex

Increased muscle activity (*Beznak et al* 1943) oxygen lack (Sundstrom and Michaels 1941) and many other stress stimuli use up free cortical hormone after which fresh hormone is discharged followed by hypertrophy with increased production But even glucose given by stomach uses up so much corticoid that increased production follows as can be seen from changes in the lymphocytes (Dougherty and White 1944) This is certainly not stress and shows that physiological factors which stimulate carbohydrate metabolism may also lead to increased output of corticoids

There is no doubt that all these reactions are absent in the hypophysectomized animals No adrenal cortical hypertrophy can occur without ACTH But it is wrong to assume that corticoid production is only a reaction to stress that is to excessive stimulation The metabolism of rest needs corticoids also Their continuous use seems to be the stimulus for their continuous production If there is increased expenditure of energy under physiological conditions more cortical secretion is used up More is needed also for the excessively increased energy production of exhaustion or the rebuilding of destroyed tissue In these circumstances the concentration of corticoids in the blood decreases to such an extent that a powerful new secretory stimulus spreads to the adrenal cortex anterior pituitary, and hypothalamic centres quite probably to all three

The production of corticoids is thus a continuous *condition* of metabolic activity and corticoids are being continuously produced and used In the words of Ingle (1951) they play a supporting role, and not a prepotent regulatory role If I understand him rightly he denies (1951 p 668) the primary role of the adrenal cortex in

corticoids influence the activity of all the body cells Ingle (1951) has recently expressed a similar view, and believes that further advances may well come through enzyme research



Normal

Adrenal ectomized

Figure 4 Alkaline phosphatase in the epithelium of the intestinal mucosa Gomori s method (After Verzar Sailer and Richterich 1952)

(4) WHEN ARE CORTICOIDS PRODUCED?

What are the stimuli leading to the production and release of the adrenal cortical hormone? Such stimuli may act directly on the gland or indirectly

We cannot yet say whether the gland can be directly stimulated It has been asserted that changes in the Na/K ratio in plasma stimulate mineralo corticoid production by the *zona glomerulosa* and that this stimulus is operative even after hypophysectomy (Swann, 1940, Greep and Deane 1949) Kendall (1938) and Ingle (1951) have both rejected this concept Similarly it has been thought that the production of carbohydrate hormone might be stimulated by lowering of the blood sugar Miller and Riddle (1941) described cortical hypertrophy after insulin hypoglycaemia, but others were unable to find any proof for this

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stress' The anterior pituitary and the adrenal cortex have rather a homeostatic role We are thus in agreement (p 670) that the adrenal cortex is not necessary for the stress (alarm) reactions but for the state of the tissues in which these reactions occur

There is another important point which requires consideration Ingle (1951) and others have already emphasized the fact that so far only *one* ACTH has been demon strated Hypopituitarism leads to decreased metabolism of proteins and carbo hydrates, as well as electrolyte disturbance Bush has shown that stimulation of the adrenal cortex with ACTH leads to increased production in unaltered proportions, of the normally occurring 11 oxycorticoids However, Selye (1951) still adheres to his views concerning mineralo and gluco corticoids, and traces the control of their secretion to the pituitary The somatropic hormone of the anterior pituitary (STH) is said to stimulate mineralo corticoid activity while ACTH controls gluco corticoids (See also the discussion by Conn Sprague and Bauer in the *Symposium on Steroid Hormones,* 1951)

There are many ways in which the adrenal cortex interacts with other endocrine glands The depressant action on the thymus—as well as lymphoid tissues generally —is one of them, while the inhibition of the thyroid with its intense catabolic activity is another A full analysis of the problem of the adrenal cortex would involve detailed consideration of these and numerous other activities

The basic characteristic of life is the power of adaptation to continually changing environmental stimuli, ranging from those producing a physiological response to others leading to exhaustion and tissue destruction An organism with inadequate production of corticoids is basically ill, and cannot adapt itself normally Just as the adrenal medulla is not the gland for emotional stress , as was believed thirty years ago so the cortex exists not merely for stress reactions, but for the maintenance of normal cell metabolism, and in this way subserves the capacity for adaptation of the whole organism

In my opinion the main beauty of this field of work is that because of the funda mental nature of the action of the adrenal cortex, we are being led to a synthesis and correlation of a number of metabolic processes of the body which were formerly not related to each other

An attempt has been made in this communication to outline such a synthesis Many problems have been touched upon both solved and unsolved I conclude by hoping that the experimental biologist should not be led away by the attraction of dialectic solutions, but should continue along the old and hard, but much safer road of experimentation

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Discussion

Chairman H Heller

J Groen Almost two years ago Professor Borst and his co workers demonstrated that extract of liquorice contains a substance which works like desoxycorticosterone in normal individuals Since then we have proved that because of this property extract of liquorice can be used in the treatment of patients with Addison s disease We have now four cases that are being kept in electrolyte equilibrium by the use of liquorice only, and we have been able to show that the active principle is *glycyrrhinic acid* This is a glucoside consisting of two molecules of glucouronic acid and one molecule of a triterpene called *glycyrrhetic acid* The physiological action in man is entirely comparable to that of desoxycorticosterone it produces sodium and chloride retention and potassium excretion If given in too high amounts the patients put on weight, develop pitting oedema hypertension increased venous pressure and may even go into paroxysmal nocturnal dyspnoea There are however two differences (1) liquorice and its component are active when given by mouth, whereas DCA is not, and (2) these substances do not seem to work in the rat whether normal or adrenalectomized

These substances act only on electrolyte metabolism They have none of the properties of glucocorticoids, and have no influence on the nitrogen balance or on the eo inophilic cells nor do they produce a remission in rheumatoid arthritis

G A Overbeek If desoxycorticosterone acts by being transformed into other substances why is it so much more active than many of them?

DCA only has an effect on glycogen storage when it is given over several days during which time the rats have to be fed is the glycogen storage not just due to intestinal absorption of carbohydrates?

F Verzar The transformation of DCA to other steroids is slow and there is probably also a summation effect The carbohydrate effect is slow in starting and is long lasting

As regards glycogen storage being a feeding effect we cannot keep these adrenalectomized rats alive by forcibly feeding them with glucose!

J F Tait The results of a bioassay developed by Mrs Simpson and myself at the Middlesex Hospital Medical School has some bearing on Professor Verzar s paper This method specifically tests the action of corticosteroids on the mineral metabolism of adrenalectomized rats (*Lancet* Jan 19th, 1952)

The collection period was very short leading according to Professor Verzar s theory to the maximum effect of such steroids as cortisone Although our results agree qualitatively with his theory in so far as cortisone acts in the same direction as DCA and adrenal extract, the quantitative results show that the specific activity of cortisone is only about 6 per cent of that of DCA and is much lower than even crude adrenal extract This extract fractionated by Zaffaroni s method of chromatography shows all the mineral activity coincident with cortisone for short runs However if the

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H Heller I should like to ask Dr Nelson what the state of the animals in his experiments was as regards anaesthesia

D H Nelson (1) The dogs were under a general anaesthetic, (2) The cow had local anaesthesia only and appears to be perfectly happy, (3) The patients were at rest in bed in some cases and in others ambulatory

P Fourman Admittedly one hormone may account for both mineral and carbohydrate effects and both effects can be shown with Compound F But it is worth recalling the difficulties in accepting this view

(1) It is doubtful whether doses of glucocorticoids adequate to control carbohydrate metabolism in Addison s disease will maintain salt balance,

(2) The amorphous fraction has the most potent effect on sodium metabolism and it is distinct from the glucocorticoids

(3) Dean and Greep s work on the *zona glomerulosa* of rats is difficult to get round it may not be applicable to man but Harrison is said to have confirmed their work in some other animals

H Heller I would like to draw attention to a recent paper by Roberts and Pitt who have shown that large doses of cortisone can maintain the sodium balance

L Weissbecker If one injects Addisonian patients with DCA there is an increase in the mineralocorticoids in the urine but there is no increase of the glucocorticoids so there must be some conversion disturbance in the body related to the insufficiency of the adrenals

F Verzar I have no experience with humans, but it is possible to maintain the sodium and potassium balance in animals if cortisone is given in small doses every six hours

chromatogram is run for longer distances, the activity completely separates from the cortisone and does not give the typical chemical tests for this steroid

Although there is undoubtedly a highly active mineralocorticoid present in extracts, its secretion by the gland still remains to be proved

D H Nelson Dr Samuels, Dr Reich and I have been attempting to determine the blood content of adrenalcortical steroids, particularly in the dog We have found three fractions I, II, III, all of which show absorption at 240 mμ and are probably therefore α, β unsaturated steroids We have not been able thus far to get any further with fractions I and III from the dog but we found that fraction II contained large amounts of Compound F, and we have later found it also contains Compound B and probably A Bush has isolated Compound E from adrenal venous blood in small quantities, as well as Compound F and Compound B

The amount of these steroids in the adrenal venous blood of a dog are of the order of 10–20 γ/ml of plasma With the help of Dr Gassner we have cannulated the suprarenal vein of cows under local anaesthesia and obtained some gallons of adrenal venous blood We have isolated a fraction from this blood which shows definite andro genic activity by the chick comb growth method There are compounds in this frac tion which give a positive Zimmerman reaction for 17 ketones show 240 mμ absorp tion for α, β unsaturated compounds, and also by infra red have free hydroxyl groups present We have however not been able definitely to assign a structure to the andro genic compound which is being secreted

We have carried out a number of studies on blood levels of 17 OH corticosteroids in humans By our method we find that normal levels are approximately 5–20 γ/100 ml plasma A single injection of 15 mgm of ACTH will cause a rise to levels of 30–40 γ per cent in one to two hours Continuous intravenous ACTH injections will raise the blood levels to 80–120 γ per cent in twenty four hours Patients with Addi son s disease consistently show no 17 OH corticosteroids in their plasma and do not respond to ACTH Three patients with Cushing s Syndrome have had values ranging from 35–80 γ/100 ml plasma The highest value was found in a man who had an adrenal carcinoma

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Control of the Secretory Activity of the Adrenal Cortex

with special reference to the isolated preparation

by

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THE role of the adrenal cortex in homoeostasis poses the question as to what enables this gland to raise its production of hormone whenever an unfavourable environment or some internal disorder requires the increased activity of one or many of the organs of the body Since we know that the rate of cortical secretion is decisively controlled by the activity of the anterior pituitary there are two aspects of that question

(1) what is the nature of the stimuli which activate the release of ACTH by the anterior lobe and is there one or are there several such stimuli?

(2) does the adrenal cortex when deprived of pituitary control, secrete at all and if so is it capable in any way of adapting its secretion to the demands of the organism?

An examination of the conditions causing a release of ACTH reveals that the majority of these so called conditions of stress which provoke the release of ACTH are accompanied by a stimulation of the splanchnic nerves and thus a secretion of adrenaline (and *nor* adrenaline) from the adrenal medulla This activity of the splanchnic nerves cannot be without effect on the cortex since intravenous injection of adrenaline in doses of the same magnitude as those liberated on splanchnic stimulation accelerate cortical secretion This was demonstrated in the dog the cat the rat and in man (Vogt 1944 Long and Fry 1945 Recant *et al* 1950) The effects are rapid large and long lasting and are presumably produced by a release of ACTH since they disappear after hypophysectomy Is however circulating adrenaline essential for the release of ACTH in conditions of stress? The question can be answered by experiments on animals in which the adrenals have been either denervated or demedullated The latter procedure consists in bilateral enucleation of the adrenal glands If a sufficient interval is allowed to elapse after the operation, there is regeneration of the cortical tissue in the complete absence of medullary cells Such experiments have shown that stimuli like hypoglycaemia or haemorrhage exposure to heat or cold injection of histamine or of β tetra hydro naphthylamine cause a release of ACTH whether adrenaline is being released or not (Gellhorn *et al* 1949 Gershberg *et al* 1950 Gordon 1950 Recant *et al* 1950 Vogt 1947 and 1951a) Under these conditions therefore adrenaline though it contributes to the secretion of ACTH by the anterior lobe is not an essential link in the chain of events constituting

We do not know whether the three mechanisms circulating adrenaline stimulation of hypothalamic centres and metabolic changes have a final pathway in common Experiments on transplants (Fortier, 1951) and experiments on animals with hypothalamic lesions (Hume 1951) can at present not be reconciled and further evidence will have to be awaited Fortier s suggestion however that only emotional stimuli produce their effect via the hypothalamus whereas all other stimuli act on the anterior lobe directly since they remain effective in pituitary transplants possibly provides the right answer

Let us now consider the second question and ask what duties the adrenal cortex can perform when deprived of pituitary control In this field care has to be taken to avoid generalizations since there exist considerable species differences Whereas for instance it is true of the rat that hypophysectomy abolishes the response of its adrenal cortex to damage by formalin or to injections of insulin this is not so in the pigeon in which hypertrophy of the adrenal can be induced by these same stimuli after extirpation of the pituitary (Miller and Riddle 1942) Differences are also obvious when the speed of atrophy of the adrenal cortex following hypophysectomy is compared for example in the rat and in the dog it takes about four times as long in the dog as it does in the rat for the same degree of atrophy to develop

The observations on this question (Pickford and Vogt 1951 Vogt 1951c) which form the remaining part of this talk were all made on cats and dogs One series was made on perfused glands the left or both adrenals being isolated and perfused with blood from a Dale Schuster pump In other experiments adrenal blood was collected *in situ* from the otherwise normal or from the hypophysectomized dog

Assay of cortical hormone was carried out in the adrenal effluent by injecting it into adrenalectomized rats exposed to low temperature Fig 1 shows a comparison of cortical secretion per min and per g adrenal by adrenal glands *in situ* and isolated perfused glands The unit is arbitrary representing the activity of 1g beef gland extracted by a commercial process for the preparation of medicinal adrenal extracts All figures are only approximations since the potency of the extracts is not the same throughout all assays nor is the assay method very accurate The results of the comparison depend on the state of the gland *in situ* chosen for comparison If as was done in the experiments of this table the secretion is measured after dividing the splanchnic nerves and allowing an interval of not less and preferably more than thirty minutes to elapse till sampling is begun there is very little difference between the secretion rate of the gland *in situ* and that of the perfused gland which is deprived of any control by the pituitary The secretion from an isolated dog s adrenal remains constant for about two hours if continuously supplied with fresh blood In terms of Compound E its secretion per g tissue and per min is about 4 μg (Bibile 1950) Addition of ACTH to the perfusing blood greatly increased the hormone yield Does this mean that the dog s adrenal will secrete in the absence of ACTH? In order to establish this point the presence of ACTH in the perfusing blood has to be excluded This was done in the experiments marked H by perfusing with blood collected from a hypophysectomized donor Later however it was learnt that this precaution may have been unnecessary since in all experiments the perfusing blood was withdrawn from the animal and circulated in the pump for about two hours before obtaining the samples and ACTH probably does not keep in blood under these conditions

the adreno cortical response to stress Its contribution to the response varies with the type of stimulus Thus it is negligible if an animal is exposed to cold environmental temperature, but large in the trauma of an abdominal operation it is easy to demonstrate that the rate of cortical secretion is higher during an abdominal operation in which the splanchnic nerves have been left intact than if they were cut at an early stage of the operation In emotional stress, the part played by adrenaline is very large indeed In fact, it has been suggested (Gershberg *et al*, 1950) that release of ACTH in emotional stress may be entirely caused by circulating adrenaline This is however, an over simplification The adrenal cortex of the demedullated rat accelerates its secretion during emotional stimuli, though its response is greatly diminished in comparison with the normal gland Neither need the emotional stimulus be severe in order to elicit the response the mere taking of the rectal temperature in rats unaccustomed to this procedure will release ACTH in the demedullated animal (Vogt 1951b) The mechanism involved is probably that demonstrated by de Groot and Harris (1950) in the rabbit In this animal, an emotional stimulus causes a lymphopenia which is the expression of an accelerated secretion of cortical hormone following the release of ACTH Localized damage to the posterior part of the hypothalamus abolishes this reaction, whereas electrical stimulation of that same region produces a lymphopenia The adrenal medulla or the sympathetic chains are not involved in that response, so that it follows that the excitation of certain diencephalic centres represents a second mechanism which is able to release ACTH

Emotional stimuli can obviously not be made responsible for the majority of adrenal responses to stress, and we have to look for yet another factor which might activate the anterior lobe whenever the body is subjected to some damaging agent Sayers and Sayers (1947) have suggested that increased utilization of cortical hormones by the stressed tissues reduces the blood levels of these hormones and that this low level of circulating corticoids is the stimulus causing the cells in the pituitary to produce ACTH This attractive theory is founded on the observations made by Ingle and by the Sayers that excess cortical hormone, administered parenterally, will depress the secretion of ACTH under conditions of stress It has however, been much more difficult to provide evidence for a *stimulation* of pituitary activity by *low* levels of circulating hormone In one extreme condition such evidence has recently been obtained by Gemzell, van Dyke Tobias and Evans (1951) These workers were able to demonstrate increased blood levels of ACTH in adrenalectomized rats which were, of course, completely lacking in circulating cortical hormone On the other hand estimation of cortical hormone in peripheral blood in man by Nelson *et al* (1951), did not provide any evidence of the hypothesis put forward by Sayers and Sayers In moribund patients, in fact the blood levels of cortical hormone went up, not down It may, of course be that under those conditions the regulatory mechanisms are no longer operating normally or that the techniques are not sensitive enough to pick up small fluctuations in hormone concentration

Though it is not certain whether the stimulus causing acceleration of the release of ACTH is in fact, a fall in the level of circulating cortical hormone, it is generally agreed that some metabolic disturbance must underlie all those conditions in which, even in the absence of emotion or adreno medullary secretion the pituitary releases ACTH

ACTIVITY PER MIN PER g ADRENAL

IN SITU		PERFUSED		
No	·g GLAND	No	g GLAND	
67	3 3	176	4 6	N
71	5 0	194	5 4	N
57	5 7	130	6 2	N
69	6 0	262	7 5	N
64	6 8	133	8 9	N
77	7 3	212	3 4	H
73	15 0	210	8 0	H
		261	10 0	H
M	7 0	M	6 75	

Figure 1

Another factor found to influence the secretory rate of perfused adrenals was a change in the Na/K ratio of the plasma When this ratio was lowered from the normal figure of approximately forty five to between nine and seven acceleration of cortical secretion occurred in the perfused gland The decisive factor in such experiments might be the lack of Na the excess K an additive effect of these two factors or the altered Na/K ratio Since the result of adding KCl was the same as that of replacing some of the sodium by potassium whereas the substitution of sucrose for Na did not change the rate of secretion of the cortex the effect must have been due not to Na lack but to excess K or to the decreased Na/K ratio

Has this phenomenon any physiological implications? The fact that regulation of the mineral metabolism appears less disturbed by hypophysectomy than by adrenal ectomy and the production of histological changes in the adrenal of the hypophysectomized rat by a sodium deficient diet (Deane *et al* 1948) would tempt us to such a conclusion and to the assumption that changes in the blood electrolytes have a direct effect on cortical secretion The necessity however to reduce the Na/K ratio to figures as low as from 7–9 in order to obtain effects on the perfused adrenal renders this interpretation doubtful Such a low Na/K ratio does not occur *in vivo* except in adrenalectomized animals so that these experiments cannot be considered to prove the physiological importance of a mechanism which may only be called into action under extreme pathological circumstances

Of the drugs tested only histamine was occasionally found to accelerate the secretion of the perfused adrenal Because of the inconsistency of this effect however it is likely that under natural conditions circulating histamine acts entirely by means of the release of ACTH

Hechter (1949) has reported that perfused beef adrenals secrete practically no hormone unless ACTH is added to the blood. The difference between Hechter's results and those on perfused dogs' glands are most likely not due to the use of another species but to differences in perfusion technique, since the possibility of avoiding even temporary anoxia when perfusing a dog's gland should contribute greatly to its better functional performance.

Since acute deprivation of ACTH does not interrupt the secretory activity of the adrenal cortex of the dog, it was of interest to investigate the effect on cortical secretion of hypophysectomy performed days and weeks before collecting adrenal venous blood. In hypophysectomized dogs which were not maintained on ACTH, cortical hormone was invariably found in the adrenal effluent. The tests were carried out up to one month after hypophysectomy.

It follows from these observations that the dog's adrenal secretes measurable amounts of corticoids whether acutely or chronically deprived of ACTH. Is such a gland, however, still capable of adapting its secretion to the varying needs of the body? Since the adrenal cortex has no nerve supply, any control of its speed of hormone production would have to be effected by changes in some constituent of the blood which might act as a stimulus to cortical secretion. A number of metabolites were tested for such activity by adding them to the blood supplying a perfused adrenal. In the same way a few drugs were tested for a possible action on cortical secretion.

The substances examined on the perfused preparation were:

Glucose	Adrenaline
Sodium lactate	Nicotine
Glycine	Histamine
Alanine	Colchicine
Cystine	Morphine
Sodium ascorbate	
Organic and inorganic phosphates	
Adenosine	
Na and K	

Of the metabolites only certain phosphoric acid esters produced an increased output of corticoids. This increase outlasted the period of injection by about fifteen minutes. Adenosine triphosphate and creatine phosphate were both active, whereas adenosine, adenosine di-phosphate and sodium phosphate were not. Since the adrenal cortex contains practically no store of hormones but has to manufacture its secretion as it pours it into the blood, its oxygen uptake and energy requirements are high and it is perhaps the supply of readily available energy which accounts for the acceleration of secretion by arterial infusion of those esters which carry high energy phosphate bonds. Support for this hypothesis is given by the work of Gemzell (1948) who found that ATP is present in normal adrenals and that the administration of ACTH causes an increased rate of uptake of P^{32} by the adrenals of rats injected with $Na_2HP^{32}O_4$.

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Discussion

Chairman S Zuckerman

F Verzar The adrenalcortical hormone is secreted continuously as a condition of normal metabolism and not only in response to stress Adaptation is the most normal function of life It is not only under conditions of stress that one needs the suprarenal hormone and ACTH they are needed continuously for normal metabolism For instance if one feeds a rat one gram of glucose one gets also a lymphopenia lasting for many hours

M Vogt After all every meal is a stress of a kind

G D Kersley Dr Vogt mentioned stimulation of the pituitary by hypoglycaemia to cause increased production of ACTH I have attempted to use this in rheumatoid arthritis 40–50 per cent of the patients showed definite improvement and all these showed an eosinopenia How does this work?

We took two groups of patients with rheumatoid arthritis and treated them by insulin hypoglycaemia and allowed them to improve to their maximum extent Then in the first group we gave 60 mgms cortisone a day the cortisone being given after they had recovered from their daily hypoglycaemia All these cases showed further improvement In the second group we carried on with the insulin giving the cortisone at the same time so that there should be no corticoid deficiency during the hypoglycaemia All these patients deteriorated

This evidence suggests that the stimulation of the pituitary by insulin hypoglycaemia is due to reduction of the circulating corticoids

M Vogt There is some recent American work that insulin and cortisone have no effect when they are given together

A V Neale I would like to ask Dr Vogt whether she has found any substance which diminishes secretion of cortical hormones In particular what is the effect of anaesthetics etc ?

To sum up, the perfused adrenal carries out its synthetic activity efficiently, but at a steady rate The only physiological mechanism found to accelerate its secretion is the addition of ACTH to the perfusing blood Other means found capable of stimulating the output of hormone from the perfused gland appear too drastic to be likely to be of physiological importance

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and had an associated low blood potassium and chloride We wondered whether the high blood corticoid level was associated with a rather severe alkalosis resulting from the electrolyte changes We administered potassium chloride to one of these patients and found that in two days the blood corticoids fell to normal levels and the patients showed marked clinical improvement These are the only untreated patients we have seen with high cortical steroid levels with the exception of terminal cases who generally show increased levels of these steroids Patients with acute pneumonias and other infections do not generally have increased levels of 17 OH cortical steroids

H Dale Is there any possible direct connection between the pituitary and the hypo thalamus which would allow of the intervention of adrenergic substances?

M Vogt Hume and Wittenstein tried to produce eosinopenia by the administration of adrenaline to animals with hypothalamic lesions and failed We do know of course that there is a high concentration of sympathin in the hypothalamus but its role is as yet uncertain

S Zuckerman I believe Hume and Wittenstein were trying to make lesions in the tuber cinereum Sayers obtained positive responses with the anterior pituitary trans planted to the anterior chamber of the eye

M Vogt I agree, but to get an effect on the transplants via the blood stream would require the release of greatly increased amounts of the active material The trans plants are far from normal as they do not adequately maintain the gonads and the adrenals This renders interpretation of the results on transplants difficult

D J Ingle I have been properly criticized in the past for ignoring the fact that one can never be certain that one has removed all the secreting cells of the pituitary This can be said of any endocrine gland extirpation experiment It is true that in the dog in order to get suprarenal atrophy one has to remove the tuber cinereum and in fundibulum along with the pituitary

I am not impressed with the role played by adrenaline in adaptation as one can get reasonable adaptation in the demedullated animal In conditions under which adrenaline will cause adaptation phenomena in the anterior pituitary adrenal cortex axis other substances will produce the same results e g both adrenaline and hista mine affect the transplants of pituitary in the eye

With regard to the adequacy of the pituitary transplants I was successful in carry ing this out in 1936 and the animals remained alive for many months I have also transplanted the pituitary under the capsule of the ovary and this seems to be a better site The suprarenal cortex in these animals was partially maintained but did not react to stress Recently several other investigators have made transplants into the eye and found that the response of the suprarenals to stress as measured by the as corbic acid depletion method was maintained, but this is not wholly convincing as there were no concomitant morphologic changes in the suprarenals

Harris showed recently that when the pituitary transplant was remote from the hypothalamus the suprarenal cortex was poorly maintained but when it was placed under the hypothalamus so that vascular connections were established between the graft and the hypothalamus the suprarenal again reacted normally to stress

M Vogt I know of no substance which will cause a diminution of secretion of the cortical hormones Inhalation anaesthetics increase this secretion whilst intravenous barbiturates are without effect

S Zuckerman I was interested to hear about Professor Verzar's views on conversion of one steroid to another, and I wonder Dr Vogt, if in your experiments you always got the same substances secreted by the isolated suprarenal whatever the stimulus, or do different stimuli give different results?

M Vogt Bibile and I tried to stimulate the isolated suprarenal with potassium chloride and ACTH The difficulty in answering your question arises from inaccuracy of the bioassays We used two techniques, (1) the eosinopenic test which we thought should test for the 11 oxy corticoids, and (2) the cold test which is also sensitive to small doses of DCA and should therefore show up DCA like mineralo corticoids if such occur naturally We did find that these two tests gave different results to DCA but with our perfusates the results of the two tests always correlated well

The order of magnitude of the steroid secretion by the isolated gland was the equivalent of four micrograms of Compound E/min/grm of gland

J M Yoffey One of the perplexing anatomical problems is why the cortex and medulla are so closely related, it seems difficult to regard this as a purely accidental association I believe it was Cramer who did some work on the anastomoses between the medullary sinusoids and the capillaries of the reticularis and suggested a functional implication

Can Dr Vogt throw any light on the fact that the demedullated cortex responds to cold and hypoglycaemia to a lesser degree than in the normal animal?

M Vogt The comparison of cortical response before and after demedullation or denervation is rendered difficult by the fact that by depriving the animal of the adrenaline normally released by cold or insulin we make what appears to be the same stress very much more severe With the depletion of the sudanophilic cortical lipoids as a test for cortical activity before denervation a great depletion of these lipoids occurred in response to insulin, but after denervation there sometimes was much less depletion This was only a qualitative test For quantitative purposes the adrenal ascorbic acid depletion test was used, but this unfortunately will not answer Professor Yoffey s question as mere fasting caused depletion of the adrenal ascorbic acid in the demedullated (and not the normal) animal and so the baseline for quantitative comparison was changed In the *denervated* gland the ascorbic acid depletion after insulin was equal to that of the normal gland

J A Lock The giving of 1 grm of glucose to a rat is equivalent to giving a man about 350 grm of glucose on the basis of body weight I should think that this is a considerable stress What happens when one administers a more normal dose?

F Verzar The rats in our laboratory eat about 10 grms of food a day so I do not think that 1 grm of glucose in about 2 cc of water is an abnormally large amount

D H Nelson During our investigations of the blood levels of 17 hydroxycorticosteroids, we came across two patients who while under the stress of a severe infection, had very high blood levels of corticoids similar to the levels one finds in Cushing s Disease

The Adreno-Genital Relationship

by

S ZUCKERMAN,

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The first indications that the adrenal glands may undergo changes in relation to the reproductive cycle and correspondingly that changes in the adrenal glands may affect the accessory reproductive organs and secondary sexual characters were provided by morphological and clinical studies more than fifty years ago.* Since then we have learnt that the changes which occur in the adrenal cortex in parallel with the phases of the oestrous cycle affect not only its total size but also the nature of its cells. We also know about the existence of sexual differences in the size of the adrenal cortex about changes in the cortex after gonadectomy and about the presence of a so called x zone in certain species. It has also become obvious that under certain conditions the gonads secrete substances which affect processes normally associated with the secretions of the cortex and *vice versa*.† The extensive literature dealing with this overlap in endocrine function has been adequately summarized by a number of workers including in the years since the war Goldzieher (1945) Parkes (1945) and Burrows (1949). The facts pose an extremely complex problem even though—as Wiesel first pointed out in 1899 and as Vannini (1951) has forcibly argued recently—it is not surprising that there should be a functional interrelationship between two secretory structures that are embryologically derived from contiguous parts of the intermediate cell mass.

The straightforward subdivision of the problem into such compartments as (*a*) the assumed production of cortical hormones by the gonads (*b*) the apparent production of gonadal hormones by the adrenal cortex and (*c*) the presumed overlap in the function of gonadal and cortical steroids provides a useful catalogue of the main facts of the interrelation. But two essential questions dominate any attempt to *understand the catalogue. They are first the possibility that the trophic control* exercised by the adenohypophysis over other endocrine organs may not be as specific physiologically as would be indicated by the behaviour of such pure chemical preparations as ACTH gonadotrophin thyrotrophin etc in experimental tests and second the likelihood that biologically active steroid molecules may undergo very rapid metabolic changes in the body with the possible consequence of alterations

* E g Stilling's (1898) observation that the adrenal glands of the pigeon increase in size at the time of ovulation and Bulloch and Sequeira's (1905) suggestion that the condition we now recognize as the adrenogenital syndrome was probably related to tumours of the adrenal cortex or to the presence of adrenal rests in relation to the male accessory glands

† E g the influence of gonadal hormones on electrolyte metabolism the influence of the corpus luteum on the survival time of adrenalectomized animals the apparent production of androgen by the cortex

F Verzar We always test for remains of pituitary after hypophysectomy by measuring the uptake of radioactive iodine by the thyroid and discard any animals that show iodine concentration

M Vogt Only a minute part of the dog s anterior lobe or of the *pars tuberalis* will maintain the suprarenals in a normal condition

S Zuckerman If we have to remove the *pars tuberalis* for complete hypophysectomy it will be quite impossible in rats

whatever its value to, say taxonomy or comparative anatomy, there could be little functional meaning to the anatomical study of an arbitrary fragment of a humerus The endocrinologist who is not a chemist needs to be assured that the specific and separate chemical substances which are defined for him as hormones do not, in effect correspond to such arbitrary fragments

At the outset we should therefore come to some agreement about the criteria by which we define the specific nature of the pure hormones now being identified by chemical methods I should like to suggest here that the fundamental consideration is that of biological function Our knowledge of any hormonal response necessarily begins with the recognition of a physiological reaction in which a tissue suspected to have endocrine function exercises an effect on some target organ As our under standing increases the facts of the physiological reaction become clearer and so, too the chemical characteristics of the hormonal substance or substances that are involved The behaviour of a purified chemical extract can be taken as representative of some physiological hormonal reaction when the two are identical Thus we equate the function of the adrenal medulla with that of the effects of an injection of say synthetic adrenalin and noradrenalin because in so far as can be determined the effects of the injection are identical with the function of the medulla as determined in physiological experiments Moreover in this instance we find no difficulty in attributing multiple effects (e g vasoconstriction and glycogenolysis) to the action of the same molecule It seems to me axiomatic that the specification of a hormone must, in the first instance be based upon an appreciation of the integrated physio logical response for which it is responsible

Yesterday Dr Nelson stated that after the administration of ACTH to a cow the blood flowing from the adrenals contained an androgenic substance whose chemical nature had been identified Professor Young in referring to this observation gave as his opinion that it constituted more convincing proof for the view that the adrenal cortex can elaborate androgen than any he had previously heard I find this difficult to understand for it makes me ask in what logical sense the important evidence pro vided by Dr Nelson differs from the kind that was already available Dr Nelson s new information as I see it relates to the chemical identity of the substance in the adrenal venous blood—a substance which he was able to show was similar to previ ously defined androgens Biochemists however have not yet ascertained the parti cular features of a molecule which underlie androgenic function And since biochemi cal knowledge is not in this sense predictive Dr Nelson s inference that the substance he isolated was androgenic derives essentially from the biological definition of an androgen (e g a substance which stimulates growth and function of accessory repro ductive organs of the male)

I have chosen this example to illustrate the thesis that the criterion by which we judge the purity or specificity of a given hormone must in the final analysis be bio logical in nature ACTH whatever its chemical structure is recognized as the adrenocorticotrophic hormone by virtue of its biological properties and it will con tinue to be so defined until such time as the chemists can explain to us the molecular characteristics upon which the adrenocorticotrophic function depends I take it too that this general proposition applies equally to gonadotrophins sex hormones and corticoids and that the concept of hormonal specificity implies that tissue differentia

in their physiological function It is to the former question in particular that the present paper is addressed

THE DEFINITION OF SPECIFICITY

The question of the chemical specificity of pituitary hormones was an underlying theme of the papers and discussions of yesterday morning, and endocrinologists of all shades of opinion cannot but be affected by the views of biochemists on this problem Yesterday s papers were essentially concerned with revealing to us chemical pieces of the jig saw we are trying to fit together at this meeting and to indicate the separate identities of various hormonal substances Dr Li referred to hormonal contaminants of ACTH , and provided evidence to show that the ascorbic acid depleting factor was something distinct from the pituitary factor responsible for the growth of the adrenal cortex The idea of specificity, although not necessarily the same as that propounded by Dr Li, was implied in Professor Young s observation about a small highly active fraction (ACTH) which 'pre existed in separable form in crude corticotrophin And Dr Morris s view is that the problem of the unity or multiplicity of adrenocorticotrophic hormones can only be settled by the isolation of specific chemical substances

It seems to me essential that endocrinologists who are not chemists should appreciate the wider implication of certain aspects of the chemical approach I say this because of the danger that in the final analysis, the isolation of a pure chemical substance which exercises specific effects may represent so great a disarticulation of an integrated physiological reaction that the specific effect or effects by which the pure substance is biologically defined may, in fact, never occur in isolation in the body To take a specific example I feel that we have to ask ourselves whether the ascorbic acid depletion of the adrenal cortex is a process distinct from stimulation of the whole cortex or merely a phase of such a stimulation—which as I understand it, is a view favoured by Sayers (Sayers and Sayers 1948) If the former it is obviously profitable to search for separate hormones mediating the different functions If the latter it becomes necessary to decide what biological significance should be attached to the individual adrenocorticotrophic hormones with which the chemist may provide us in so called pure form *

These considerations are in no sense opposed to the view that an understanding of a process as a whole necessitates a knowledge of its separate parts or stages The contribution of the biochemist is here similar to that of the anatomist when let us say he studies the characters of a bone disarticulated from the limb of which it is a part The muscular control of the movements of the arm could never be fully understood unless the anatomical characters of the humerus had been properly defined On the other hand the anatomical characters of the humerus have no functional significance except in relation to the movements which the arm can make Moreover

* It s conceivable that purification of a hormone may in some cases have great practical pharmacological importance at the same time as it does not lead to any great advance in physiological knowledge Here it is worth noting that the purest preparation of FSH which has so far been described by Dr Li (Moon and Li 1952) is much less effective biologically weight for weight than the impure preparations normally used Thus whereas as much as 5 mg of the pure preparation was necessary to enlarge the ovaries of test mice significantly an equivalent increase was obtained in rats with as little as 0 01 mg of the FSH described by Fraenkel Conrat Simpson and Evans in 194[illegible]

sense that androgen 'damps down' the adrenocorticotrophic as well as the gonado trophic function of the adenohypophysis and by so doing leads to involution of the adrenal cortex

THE TROPHIC CONTROL OF THE ADRENAL CORTEX

There is the further possibility that the indirect and depressive effect is due not just to the suppression of ACTH but also of gonadotrophin and that this trophic factor also has a direct effect on the adrenal cortex This idea appears to have been first advanced by Martin (1930 1932) who suggested that the disappearance of the x zone as a result of the administration of gonadal hormone is due to the suppression of the gonadotrophic as well as the adrenotrophic potency of the anterior pituitary the implication being that the x zone of the adrenal cortex is under the control of the same trophic factor(s) of the pituitary as are the gonads This view has been given a considerable amount of substance lately as a result of an extensive cyto chemical and experimental study by Jones (1949) According to this worker main tenance of the x zone is a function of a gonadotrophin possibly LH in nature, the well being of the *zona fasciculata* depends completely on ACTH while the *zona glomerulosa* which persists in the adrenal cortex of hypophysectomized animals is an autonomous zone although the presence of acetone soluble sudanophilic material within it still depends on ACTH Jones s experiments were carefully planned and involved groups of intact virgin female mice hypophysectomized virgin female mice hypophysectomized virgin female mice given gonadotrophin spayed non hypophy sectomized mice given gonadotrophin intact mice given ACTH hypophysectomized mice given ACTH etc If the conclusion to which his experiments point is sustained and if at the same time we accept the view that the chemical or laboratory specificity of ACTH FSH and LH implies physiological specificity we have to infer that to some extent at least the adrenal cortex is controlled by the pituitary factor(s) responsible for the maintenance of normal gonadal function

This conclusion is not entirely consistent with certain observations on dwarf mice that have been published by Deanesly (1938a) Neither the male nor the female of this genetic strain develops an x zone and the *zona reticularis* is only a narrow band of cells around the medulla Castration, which in young normal mice leads to the persistence or reappearance of the x zone has practically no effect on the adrenal glands of the dwarf mouse even though it is followed by involution of the accessory reproductive glands Since as was shown by Smith and McDowell (1930) the under lying defect in the dwarf mouse is a hypo functioning of the adenohypophysis *in toto* and since it is the gonadotrophic function which is the least suppressed Deanesly argues that while the absence of the x zone strongly suggests that it is dependent on some secretion of the pituitary deficient in dwarf mice it is hardly likely to be the gonadotrophic hormone

In spite of this observation the facts before us suggest first that gonadal hormone (e g androgen) may have a direct and stimulating effect on the adrenal cortex second that at least part of the adrenal cortex (i e the x zone) may be under the con trol of gonadotrophic hormone and third that this region of the adrenal may also be under the control of a second pituitary secretion which by definition would not be gonadotrophic

tion, in the embryological sense, is associated with a competence to react specifically to specific hormonal substances Where a tissue has no specific competence in this sense, there is no specific hormonal action

THE DIRECT AND INDIRECT EFFECTS OF ANDROGEN ON THE ADRENAL CORTEX

Any attempt to elucidate the problem of the interaction of the pituitary gonadotrophins and ACTH must begin with a consideration of the effects which gonadectomy and the sex hormones have on the adrenal cortex Here our knowledge derives mainly from work on the rat, and the central facts appear relatively straightforward Thus the removal of the ovaries in the mature female leads first to a transitory increase and then to a partial involution of the adrenal cortex In the gonadectomized male only a slight increase is observed In both sexes these changes can be prevented by means of androgenic hormone which, when given to intact female rats leads to a slight decrease in the size of the adrenal glands

These observations are in accordance with the thesis that androgen is in some way antagonistic to the adrenal cortex* and the thesis is also consistent with the well established fact that the x zone in young male mice disappears when the testis begins to secrete androgen, whereas it persists in the female until late in the first pregnancy It can also be correlated with the observation that both young male and female mice lose their x zones when treated with androgen

Until recently it was widely held that gonadal hormone exercised its effects on the adrenal cortex indirectly by way of the pituitary (e g Selye, Collip and Thomson 1935, Ellison and Burch, 1936) the implication being that an excess of androgen in the body fluids not only 'damped down the gonadotrophic function of the adenohypophysis, but also that of ACTH—the basophil cells being responsible for the production of both hormones (Marshall 1951) To day there is reason to doubt this view Thus both Cutuly Cutuly and McCullagh (1938) and Leonard (1944a) have shown that testosterone given to hypophysectomized rats partially prevents the adrenal atrophy which follows the ablation of the pituitary Leonard (1944b) has also shown that androstenediol (3 α 17 trans and 3 β 17 trans) has the same action, and that its effect is greater if the steroids are administered immediately the pituitary is removed rather than after a post operative period of ten or more days Corresponding results have been obtained more recently by Zizine Simpson and Evans (1950)

These observations suggest very clearly that androgen can directly influence the cells of the adrenal cortex and that its direct effect is that of stimulation On the other hand they do not exclude the possibility that in addition to a direct effect in some species†, a depressive effect is also mediated indirectly *via* the pituitary in the

* The results of similar experiments with oestrogen have not been as clear-cut (as a rule oestrogen leads to an increase in the size of the adrenals) and it should also be observed that all the mammalian species tested do not appear to react to castration and androgen in the same way as does the rat (e g Syrian hamster Keyes 1949 in which the adrenal cortex decreases in size after castration)

† The direct action of androgens on the adrenal cortex of hypophysectomized animals may not apply to all species Thus Zalesky Wells Overholser and Gomez (1941) found that in the ground-squirrel testosterone fails to prevent or repair the involution of the adrenal cortex that follows hypophysectomy whereas gonadotrophin will not only maintain adrenal function but even cause cortical hypertrophy

The first of these possibilities is suggested by Davidson and Moon s (1936) observation that adrenotrophic extract can bring about an increase in the weight of the accessory reproductive organs in castrated male rats Davidson (1937) later showed that this response does not occur in the absence of the adrenals and gonads and Moon (1937) made corresponding observations on immature spayed female rats whose vaginal closure membrane broke down after administration of adrenotrophic extract, whereas it remained intact in the uninjected controls Nelson (1941) also found that it is possible to induce growth in the accessory reproductive organs and mammary glands of gonadectomized rats of both sexes with adrenotrophic hormone The response occurred in the absence of the hypophysis but not in that of the adrenals In so far as gonadectomized animals were used in these experiments the implication is that the adrenals under the influence of crude adreno trophic extract will secrete a steroid which can stimulate growth in the accessory glands of both sexes Whether or not the ACTH used in the experiments was a pure preparation is of little consequence to the conclusion to which they point The essential fact is that growth in the accessory organs occurred after gonadectomy in animals given presumed ACTH The only possibility that argues against the con clusion that the adrenal cortex can produce a secretion which stimulates the accessory reproductive organs in the same way as do gonadal hormones would be that some other extra gonadal tissue has been stimulated to produce an androgen—a possibility which would seem to be excluded by Nelson s observations

The second proposition that ACTH may stimulate the gonads to produce cortical hormone is indicated very clearly by the interesting observations that have recently been reported by Clayton and Prunty (1951) and to which brief reference has already been made These workers have shown that the development of granulation tissue in mice is inhibited by preparations of ACTH that lack any detectable gonado trophic action They found that the gonads are essential if inhibition is to occur and also observed that the inhibition occurs in adrenalectomized mice born at the begin ning of the new breeding season in pregnant mice and in those treated with chori onic gonadotrophin Their view is that the gonads and adrenals are normally jointly responsible for the effect produced by ACTH whereas the adrenals are an inessential link in the chain when luteal tissue is present or when the gonads are under the influ ence of LH The implication of their experiments is that ACTH free of gonadotrophin has a direct action on the gonads stimulating them to produce some steroid substance (which probably does not fall into the class of gonadal hormones) which sub sequently exerts a specific peripheral effect on granulation tissue

The third possibility that gonadotrophin may stimulate the adrenal cortex to produce sex hormones is suggested by the fact that what may be called sub threshold oestrous cycles continue to occur in spayed female rats (Kostitch and Telebakovitch 1929 Mandl 1951) and presumably monkeys (Zuckerman 1941) Woolley (1950) has also shown that the accessory reproductive organs of cancer strain mice which had been ovariectomized soon after birth and which developed nodular hyperplasia of the adrenal cortex matured in the normal way These animals not infrequently underwent irregular oestrous cycles and they mated freely Corresponding observa tions were made on gonadectomized male mice which had also developed nodular hyperplasia of the adrenal cortex The implication of these observations is that the

These conclusions, which at first sight may appear discordant, are not necessarily incompatible Our knowledge of pituitary function has not yet reached the stage where an apparent complexity of control is suspect simply because it conflicts with the belief of interactions between *specific pituitary secretions and specific target* organs

THE INFLUENCE OF ACTH AND CORTISONE ON THE GONADS

So far I have considered only the possibility that the gonadal and pituitary hormones have a direct effect on the adrenal cortex The complementary proposition, that cortical hormones and ACTH have a direct effect on the gonads, also needs to be discussed The evidence that bears on it is slender

Part of it is summarized in a recent review by Sprague (1951), who also indicates its very equivocal nature and who suggests that such effects of cortisone and ACTH as have been observed on the gonads and accessory organs of rats and mice usually of a suppressive character may have been a reflection of the catabolic effects of the treatment rather than of a specific effect on the reproductive tract Other reviewers regard the evidence as *implying a more direct action Thus there is the* possibility that the injection of adrenal cortical hormone into normal young animals may induce precocious sexual maturity (e g Corey and Britton, 1931, who used adrenal cortical extract, and Aterman and Greenberg, 1952, who used cortisone) As no one seems to have tried the experiment either on sexually mature or immature hypophysectomized animals it is impossible to say whether the gonadal effect is direct indirect, or both On the other hand ACTH large doses of which are said to depress the testes in normal animals (Selye 1951) is reported to stimulate the preputial glands in adrenalectomized or even gonadectomized animals (Selye 1951) Clayton *and Prunty (1951) also report a number of observations suggesting that a pure* ACTH in which no gonadotrophic potency could be detected can stimulate the gonads in the absence of the adrenals (see below), while Asling Reinhardt and Li (1951) have observed that the testes may partially descend and increase in weight in immature rats treated with purified ACTH (regression occurs in uninjected hypophysectomized controls)

HETEROLOGOUS TROPHIC CONTROL

These facts about the influence of ACTH and cortisone on the reproductive organs *are clearly too few to allow of very definite conclusions The indications, however,* are that we are dealing with a set of responses which are the reciprocal of the reaction of the adrenal cortex to androgen and gonadotrophins It is therefore of interest to examine the further propositions

(1) that what we define as ACTH may stimulate the adrenal cortex not only to produce adrenal cortical hormones proper but also sex hormones
(2) that what we define as ACTH may stimulate the gonads to produce cortical hormones
(3) that what we define as gonadotrophic hormone may stimulate the adrenal cortex to produce sex hormones and
(4) that what we define as gonadotrophic hormone may stimulate the gonads to produce cortical hormones, as defined biologically as well as sex hormones

weak, and susceptible to a variety of conditions which can be tolerated by the normal individual Under Selye's influence these characteristics of adrenal insufficiency have been related to a common picture the adaptation syndrome and to the three phases which he has defined—shock adaptation or resistance and exhaustion According to Selye (1947), the whole syndrome is fundamentally related to the concentration of pituitary function on the adrenal cortex In his words there is a shift in pituitary hormone production which necessitates a decreased secretion of other hypophyseal principles in order to permit maximal corticotrophic elaboration According to him, too the alarm reaction is always associated with a decrease in the elaboration of gonadotrophin an inference which might be regarded as deriving primarily from the well known observation of the involution of the reproductive organs in Addison s disease, or in animals deprived of their adrenal glands In his most recent statement on the subject Selye (1951) reaffirms his belief in what he describes as a shift in hypophyseal hormone production as the central hormonal characteristic of the stress response

Recent observations made in my own department suggest however that not only the adrenal glands but also the ovaries increase in weight in a variety of traumatic conditions (e g surgical procedures homografting of various tissues application of corrosive fluid to uterine horn) All such conditions according to Selye s thesis would be expected to result in the secretion of excess ACTH and a decrease in the output of gonadotrophin In the rat however both the adrenal glands and the ovaries hypertrophy in these circumstances (e g after unilateral or bilateral hysterectomy [Mandl and Zuckerman 1951]) A relevant observation (Mandl and Zuckerman 1952) is that the breakdown of the vaginal closure membrane occurs significantly earlier in young rats aged twenty to thirty days exposed to cold than in their undisturbed litter mates (other less drastic treatments resulted in no such precocity) Where the breakdown occurs precociously the most likely explanation is that the pituitary is secreting an excess of gonadotrophin which in turn stimulates the production of ovarian oestrogen This inference helps to dispose of the possibility that the increase in ovarian weight after operations of the kind mentioned above is due to hydration and not to functional hyperplasia or hypertrophy of cellular elements That hydration is not the explanation is also indicated by the experiments of Swingle Seay Perlmutt Collins Fedor and Barlow (1951a 1951b) These workers subjected young rats to prolonged electrical stimulation of the *cervix uteri* a treatment which in Selye s language can hardly be regarded as anything but stress They found that this treatment in prepubertal animals accelerated the onset of sexual maturation as measured by uterine hypertrophy They suggest that the stimulus apparently causes the anterior pituitary to secrete sufficient FSH and LH to bring about oestrogen release from ovarian follicles In seven 32 day old rats the ovaries contained well developed *corpora lutea* Daily administration of dibenamine inhibited the effect of the stimulation suggesting that the pituitary of the immature rat is able to secrete LH as a result of neurogenic stimulation in the same way as has been shown for the rabbit following copulation (Sawyer Markee and Townsend 1949)

In other experiments Swingle *et al* (1951b) subjected adult female virgin rats to various types of non specific stress e g injection of fresh tissue homogenates adrenaline formaldehyde etc The uteri of these animals previously traumatized by the

adrenal cortex is able to produce hormones which by their biological action can only be defined as gonadal hormones The facts are, however, insufficient to show whether the adrenal cortex functions autonomously in these circumstances and if it does not, whether it is pituitary gonadotrophin or ACTH, which stimulates the production of sex hormones

The fourth proposition, that the gonads, under the influence of gonadotrophic stimulation can produce a hormone which by biological definition should be regarded as *adrenocortical* is suggested by the well known observation that the survival rate of adrenalectomized animals (e g rats and dogs) is prolonged when functional *corpora lutea* are present in the ovaries (i e in pseudopregnancy or pregnancy) Further proof that the ovaries can produce a substance which prolongs the life of adrenalectomized animals is provided by the experiments of Emery and Schwabe (1936) These workers removed the adrenals from two hundred rats at about the age of thirty days, and then treated them with pituitary extracts They found that normal females in which *corpora lutea* were induced survived very much longer than spayed females or males *Corresponding results have been obtained by a number of other* workers (see Burrows, 1949) While the usual interpretation of such experiments is that progesterone is the agent responsible for the prolongation of life the possibility that the activated ovaries also produce some steroid more immediately related to the secretion of the adrenal cortex cannot be excluded

The work I have reviewed so far indicates a reciprocal interaction of gonadotrophins and ACTH on the one hand and of the adrenal cortex and the endocrine elements of the gonads on the other Although we are accustomed to suppose that the pituitary controls a number of distinct functions by means of a number of distinct and specific hormonal secretions, the question that it is of interest to ask now is whether ACTH and gonadotrophins are as independent physiologically as they obviously are chemically

PHYSIOLOGICAL LINKAGE OF ACTH AND GONADOTROPHINS

I have elsewhere referred (Zuckerman, 1952) to the parallelism of gonadal and adrenal changes as they relate to the onset of the breeding period in seasonal mammals The evidence which I brought together indicated fairly clearly that the same environmental changes which stimulate the gonads are simultaneously associated with the activation of the adrenal cortex The interaction, or parallel action of ACTH and gonadotrophin is also manifested in those physiological conditions which it has become customary to describe under the general terms stress or alarm reaction as defined by Selye These two terms have in practice come to denote almost any set of conditions in which the equilibrium of the organism within its environment is altered beyond what, for present purposes can be called normal limits

THE BEHAVIOUR OF THE OVARIES IN RESPONSE TO STRESS

It has long been known that adrenalectomized animals are very sensitive to toxins, infections, various drugs anaesthetics etc in the same way as it has long been common knowledge that patients suffering from Addison s disease become extremely

addition to the removal of the adrenals had their ovarian artery and nerves divided survived until such time as the ovaries were removed, when death immediately supervened These animals did not undergo oestrous cycles after the operation, and histological examination showed the ovaries in these animals to be very small, and to possess only an occasional follicle or corpus luteum

If by adreno cortical or corticoid hormones we understand a steroid which maintains life in an adrenalectomized animal it follows that some such substance was being secreted by the denervated ovaries of Hill s mice On the other hand, it is known that progesterone will also maintain the life of adrenalectomized animals and even though the histological structure of the denervated ovaries hardly suggests that this steroid was being secreted the possibility that it was this substance which was responsible for the longevity of Hill s adrenalectomized mice cannot be excluded Other observations relating to the maintenance of adrenalectomized animals by means of progesterone are referred to below

EVIDENCE DERIVED FROM EXPERIMENTS IN WHICH HORMONES WERE INJECTED

The observations which I have reviewed suggest very clearly that the adrenal cortex and the secretory elements of the gonads are not as sharply differentiated in the functional sense as one would infer from the differences in their definitive structure—any more than they are embryologically * In his detailed review of the subject Parkes (1945) gives many other instances of the lack of specificity of gonadal tissue and the adrenal glands

Unfortunately the analysis cannot be carried further in any precise way by a consideration of the effects of injected steroid hormones We know for example that crystalline progesterone is able to maintain the life of adrenalectomized animals and that chemically pure sex hormone such as oestrone have a powerful effect on electrolyte metabolism and one which can be likened to that of the deoxy steroids But an essential question which suggests itself when we review experiments in which an overlap in function between the gonads and adrenal glands is inferred from the effects of injected hormones is whether these effects are due immediately to the steroid substances that were injected into the body or to some other steroid into which they may have been transformed Parkes (1945) makes the same point when discussing the androgens and similar substances that are found in the urine of normal and gonadectomized men and women While they may be wholly of adrenal origin he points out that there is no proof that they are secreted by the adrenal as such and that they may well be excretion products of the cortical hormones proper since there is nothing inherently improbable in the idea that gonadal and cortical hormones have common excretion products Sayers (1950) makes an identical point when he talks about the conversion of cortical hormones to androgens by the liver or other tissues

A great deal is known about the metabolism of steroid hormones and much about their rates of turnover We know many if not all, of the tissues in which the steroid molecule is metabolized and we also know that the rates at which the transformations

* Structures which have an equally close embryological relationship usually become completely differentiated anatomically and functionally from each oth e g dorsal root ganglia and adrenal medulla parathyroid and thyroid

insertion of threads, showed large, well formed deciduomata Swingle and his colla borators consequently conclude that any vigorous non specific stress is able to bring about LH release from the pituitary in rats of all ages This conclusion agrees with that derived from the experiments carried out in my own laboratory, but neither set of findings corroborates Selye s thesis that gonadotrophin output necessarily falls when excess ACTH is secreted by the pituitary as a result of 'stress

The likelihood that conditions of the kind that have been classified by Selye under the heading of stress may be associated not only with an increased discharge of ACTH but also of other pituitary factors, is supported by Brolin s observations (1946–47) that exposure to cold stimulates not only the secretion of ACTH but also that of thyrotrophin These effects are paralleled by certain cytochemical changes in, and hypertrophy of the basophil cells of the pituitary It may also be noted that Saxton and Greene (1942) have observed that, in the rabbit, the stimulus of mating results not only in a greater concentration of gonadotrophin in the pituitary but also of thyrotrophin and possibly of ACTH as well

Contrary to Selye s rather rigid formulation of a 'pituitary shift , these various observations lead to the general *conclusion that many conditions that can be* classi fied under the general term of stress may be associated with the liberation of two or more trophic principles of the adenohypophysis I am not, of course suggesting that all conditions of stress are associated with an increase in the size of the gonads My *purpose is to show that in many circumstances of stress the adrenal glands* and the gonads appear to respond in a corresponding way

THE SPECIFICITY OF THE TARGET ORGANS

The observations that I have reviewed argue in favour of the idea that under normal, and under at least some abnormal conditions, the adrenocorticotrophic and gonado trophic powers of the pituitary are not physiologically disarticulated from each other *The considerations to which I drew attention in the earlier part of this paper* suggest that these two trophic principles can, in fact, act on both sets of target organs, and further that the latter can elaborate both homologous and heterologous hormones—that is to say, the adrenal cortex can produce sex as well as cortical hormones and *the gonads cortical as well as sex hormones*

The lack of any final hormonal specificity in the gonads is even more clearly illu minated by the experiments carried out by Hill on mice (Hill 1937a b 1941 1951 and Hill and Strong, 1938) Hill first showed—and his experiments have been amply *confirmed on rats by* Deanesly (1938b) and other *workers* (e g Lampton and Miller 1941)—that ovarian grafts in the ears of castrated male mice can produce androgens and that in this respect their behaviour can be controlled by regulating the external temperature He also showed that the grafts produce oestrogenic hor mones, as indicated by the growth of the rudimentary mammary glands of the male hosts More recently (1951) he has shown that the denervated and isolated ovary of the mouse can produce a hormone which maintains life after adrenalectomy In these experiments he cut the ovarian artery and its attendant nerves and then removed both adrenal glands In control animals in which the ovarian pedicle was not touched mortality was practically 100 per cent The majority of the animals which, in

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of the molecules occur varies between different tissues and in different physiological states of the body (for example, there is a sixfold increase in the excretion of cholesterol in the bile of hyperthyroid rats Rosenmann Friedman and Byers 1951) Something is also known about the reactivation and interconversion of steroid hormones (e g Kochakian 1946 Samuels, 1949, Soffer Gabrilove and Jacobs, 1949 Hechter, Jacobsen, Jeanloz, Levy, Marshall, Pincus and Schenker, 1950, Hechter, Zaffaroni, Jacobsen, Levy, Jeanloz, Schenker and Pincus 1951) The very rapid turnover rate of intravenously injected hormones has also been studied by many workers

To one who is not a chemist, the central conclusions to which these biochemical studies seem to point are, first that a biologically active steroid may be rapidly transformed within the body to some other biologically active substance, and second, that we do not know the nature of the biologically active hormones at the cellular level

CONCLUSION

The adreno genital relationship appears to resolve itself, not into any clear picture of specific interactions but into the vague outlines of a concept whose central feature is community of control and community of response It is a picture wholly different from the view we get of the interrelationships of other endocrine organs Thus the question never arises whether the thyroid or parathyroid glands elaborate, in any physiological or pathological condition, those hormones which are produced either by the adrenal glands or the gonads any more than we find ourselves debating whether gonadotrophin or ACTH acts on the thyroid, and thyrotrophin on the adrenal glands and gonads This is not to say that under normal conditions the adrenal glands and the gonads are not distinct tissues subserving distinct functions What is implied by the facts is that these distinctive functions may be interrelated at every turn, the secretory elements of the gonads being able to serve, in certain conditions as accessory adrenal tissue and the adrenal cortex in its turn as tissue not dissimilar from the secretory tissue of the gonads Moreover, these accessory functions of the two organs appear to be controlled in the same way as the main functions to which they are secondary This seems the only view one can take in the absence of any evidence which shows that the substances which have been crystallized as gonadotrophins and the one(s) prepared as chemically pure ACTH, are in fact secreted as such, and independently by the anterior lobe of the pituitary

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The Adrenal Cortex and the Mammary Gland

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THE ADRENAL CORTEX AND LACTOGENESIS

THE pattern of the hormonal control of the initiation of lactation is more complex than was believed when the anterior pituitary lactogenic hormone prolactin was first discovered It soon became evident that purified prolactin will not by itself initiate lactation in hypophysectomized animals though unfractionated anterior pituitary extracts are effective Thus lactogenesis proved to be a change influenced by more than one anterior pituitary hormone That the pituitary adrenal cortex mechanism is also involved in lactogenesis was shown by the fact that lactation can be initiated in hypophysectomized guinea pigs by treatment with purified prolactin together with ACTH adrenal cortex extract or as eventually shown by Nelson Gaunt and Schweizer (1943) cortisone

It is for this reason that we have included cortisone among the hormones whose effects on the metabolism of mammary gland slices *in vitro* are being studied in our laboratory Respiratory studies have shown that lactogenesis as ordinarily understood i e the initiation of copious milk secretion in the rat at parturition is accompanied by an increase above unity of the respiratory quotient (R Q) of mammary gland slices in the presence of glucose or glucose + acetate (Folley and French 1949 1950) In general accord with these findings measurements of the time course of the total pressure changes (composite respiration curve) when rat mammary gland slices were incubated in bicarbonate saline in equilibrium with oxygen CO_2 and containing glucose + acetate have shown that the metabolism of slices from pregnant rats (twenty days) is such that the overall pressure slowly falls while that of slices taken from lactating rats results in a progressive rise in overall pressure (Balmain and Folley 1952) We have used this convenient technique for pilot studies of the effects of hormones *in vitro* on mammary gland slices since a change from a composite respiration curve of negative slope to one of positive slope may be expected to accompany the initiation of secretory changes in the tissue

In experiments on mammary gland slices from twenty day pregnant rats (Balmain and Folley 1952) prolactin added to the medium has so far had no detectable effect on the composite respiration curve which at this stage has a negative slope Cortisone on the other hand markedly affected the pressure curve the slices in presence of the corticoid giving a curve with the positive slope characteristic of lactating tissue This would at first sight appear to permit of the interpretation that the hormone had initiated metabolic changes in the direction of lactogenesis Addition of both prolactin

lactating rats receiving 3 0 mg DCA daily are abnormal in that the water content of the tissue is significantly greater than that of controls (Folley and Greenbaum, 1948 *a*)

The superiority of DCA over 11 oxysteroids for supporting lactation after adrenalectomy in our rats was maintained even when the dietary protein was raised to 50 per cent with a view to favouring the action of the glucocorticoids (Cowie and Folley 1947), and also in face of considerable changes in the dietary Na/K ratio (Cowie Folley, French and Greenbaum 1947 Cowie (1952) though Nagareda and Gaunt (1948) found that rather extreme variations in the dietary uptake of Na and K did alter the lactation maintaining ability of DCA in their rats However in more recent unpublished experiments in our laboratory, Dr A T Cowie (1952) has obtained virtually complete lactation maintenance in our adrenal ectomized lactating rats by administration of cortisone (implantation of 2 × 11 mg pellets yielding 0 85 mg /day) together with DCA (implantation of 1 × 50 mg pellet yielding 0 36 mg /day)

We have carried out biochemical studies with the object of learning more of the relation between the adrenal cortex and milk secretion The mammary tissue of the rat and mouse contains appreciable quantities of arginase during lactation In these species the enzyme seems to be closely related to the secretory function of the gland because in the rat the enzyme level which is low during pregnancy and lactation begins to rise at about the fourth or fifth day and thereafter increases steadily to a level some five to six times higher at the twentieth day (Folley and Greenbaum 1947) Adrenalectomy at the fourth day of lactation largely prevents this increase so that the mammary gland arginase levels of adrenalectomized rats killed in full lactation are significantly depressed compared with pair fed controls (Folley and Greenbaum 1948 *b*) The arginase levels of the liver kidney and intestinal mucosa of lactating rats are also depressed by adrenalectomy (Folley and Greenbaum 1948 Folley and Watson 1951)

Folley and Greenbaum (1947) interpreted their results on the basis that the mam mary gland arginase is part of a system which effects gluconeogenesis from protein (or amino acids) in the gland the deaminized residues being available as milk precursors or as a source of energy The rise in the arginase level of the rat mammary gland during lactation was thus believed to reflect an increase in the rate of gluco neogenesis from protein as the secretion rate increases Accepting this and given also that the adrenal cortex, through the 11 oxysteroids regulates gluconeogenesis in the mammary gland as well as the liver then the depressing effect of adrenalectomy on lactation would be mediated through the resulting decrease in the supply of deamin ized residues However the importance of gluconeogenesis in the mammary gland in animals other than the rat and mouse is doubtful since in lactating mammary tissue from herbivorous animals (guinea pig rabbit cow goat sheep) Folley and Greenbaum (unpublished) have found very little arginase and in this connection it should be pointed out that the effects of adrenalectomy on lactation in herbivorous animals are not known Moreover the actual biological role of the mammary gland arginase in the rat itself must now be regarded as obscure since we have recently found cortisone to be far more active than DCA in re toring the depleted tissue arginase levels following adrenalectomy (Folley and Watson 1951) a result which is

and cortisone had no greater effect than cortisone by itself so that no synergistic effects of the two hormones *in vitro* have yet been observed

The conclusion that the high R Q of slices of lactating mammary gland signifies that the tissue is capable of effecting net fat synthesis from small molecules *in vitro* (Folley and French, 1950), has been confirmed in experiments in which the acetate or the glucose was labelled with C^{14} and radioactive fatty acids isolated from the slices (Balmain Folley and Glascock, 1952 *b* and further unpublished work) Therefore in order further to investigate the meaning of the effects of cortisone on the composite respiration curve of mammary slices from pregnant rats (twenty days) we have studied the effects of cortisone and prolactin *in vitro* on the incorporation of C^{14} into the fatty acids of such slices incubated with [*carboxy* C^{14}] acetate + glucose In view of the respiration results already mentioned we were not surprised to find that the relatively small but nevertheless definite incorporation of $CH_3C^{14}OONa$ into the fatty acids of the slices at this stage was quite unaffected by prolactin in the medium (Balmain and Folley unpublished) Cortisone, however, significantly decreased instead of increasing the radioactivity of the fatty acids a result which was quite unexpected (Balmain Folley and Glascock, 1952 *a*) The conclusion from these preliminary results must therefore be that cortisone *in vitro* depresses the utilization of acetate carbon for fatty acid synthesis by mammary gland slices from pregnant rats

At present it is hard to reconcile this action of cortisone with its effect on the composite respiration curve of non lactating tissue and also to interpret it in relation to the intervention of the pituitary adrenal mechanism in lactogenesis It would seem that the change from a falling to a rising overall pressure curve evoked here by cortisone must be due to stimulation of some phase of mammary metabolism other than fat synthesis The lack of effect of prolactin on the metabolism of the slices at this stage is also surprising since we have found that just after parturition (days 1–5) the tissue responds to prolactin in the medium with an increase in the slope of the composite respiration curve (Balmain and Folley 1952) It may be that mammary tissue needs conditioning with corticoids before it is able to respond to prolactin

THE ADRENAL CORTEX AND THE MAINTENANCE OF LACTATION

It has long been known that adrenalectomy of lactating animals causes a marked depression of lactation Paired feeding experiments on rats indicate that though this decline in secretion rate is in part due to the reduction in food intake resulting from adrenalectomy, part must be ascribed to loss of the adrenals *per se* (Cowie and Folley 1948 Cowie, Folley French and Greenbaum, 1949) Gaunt, Eversole and Kendall (1942) reported complete maintenance of lactation in rats adrenalectomized on the day following parturition by regular administration of adrenal cortex extracts or cortisone, deoxycorticosterone acetate (DCA) being much less effective In our laboratory, on the other hand DCA (3 0 mg /day) proved more effective than 11 oxysteroids (subcutaneous doses up to 1 0 mg in oil daily) in maintaining lactation in rats adrenalectomized on the fourth day of lactation, though the restoration due to DCA was usually not complete (Folley and Cowie 1944 Cowie and Folley 1947) It is worth noting in this connection that the mammae of adrenalectomized

mammary tissue from the rat, and it is of interest that an antagonism between cortisone and insulin is apparent when both hormones are added together (Balmain, Folley and Glascock, 1952 *a* and further unpublished work)

It is hoped that these experiments which are still in progress will throw further light on the biochemical nature of the relationship between the adrenal cortex and the mammary gland It is too early to say whether this effect of cortisone is mediated through changes in the permeability of tissue cells to substrates as postulated by Elliott and Yrarrazaval (1952) for liver and brain slices, or whether the hormone more directly influences the enzyme systems concerned in lipogenesis In any event these observations may have a general significance in respect of the biological action of cortisone and other 11 oxycorticoids

For the gift of materials used in the unpublished experiments referred to in this communication I am indebted to the following Dr R W Bates of Messrs E R Squibb and Sons for purified prolactin, to Dr R T Major and Dr C C Porter of the Merck Institute for Therapeutic Research for crystalline cortisone and Compound A to Dr Abraham White of Chemical Specialities Co Inc for Compound B, and to Dr W J Haines of the Upjohn Company for Compound F

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not in agreement with the relative lactation maintaining activities of these steroids in the same rats

Since of the manifold synthetic activities of the mamma, most is known about the biosynthesis of milk fat (see Folley 1952), we have also sought for a relation between the adrenal cortex and fatty acid synthesis in mammary tissue In unpublished experiments in our laboratory, Dr R F Glascock and Mr W G Duncombe found that the ratio of the specific radioactivities of the volatile and non volatile fatty acid fractions isolated from the mammae of rats given [*carboxy* C^{14}] acetate before autopsy at the seventeenth day of lactation was *significantly increased by adrenal* ectomy at the fourth day This indication that loss of the adrenals had caused some disturbance in the mechanism of fatty acid synthesis in the mamma suggests some general relationship between the adrenal cortex and lipogenesis

Experiments on mammary gland slices from lactating rats, as well as those on slices from pregnant rats already mentioned have confirmed the existence of such a relationship We have found that in presence of cortisone the positive slope of the composite respiration curve resulting from the metabolism of mammary gland slices from fully lactating rats (substrate acetate + glucose) is markedly decreased (Balmain Folley and Glascock, 1952 *a*) an effect directly opposite to that observed when insulin is added to the medium (see also Balmain, French and Folley, 1950) In further preliminary experiments we have found that corticosterone is even more active in this respect than cortisone since it exerts a similar effect in smaller concen trations while Compounds F and A have so far proved much less active (Balmain and Folley unpublished) The insulin effect is undoubtedly a reflection of an increase in R Q which in turn stems from an acceleration of lipogenesis from small molecules (Balmain and Folley 1951, Balmain, Folley and Glascock 1952 *b*) It therefore seemed probable that, conversely the decrease in the slope of the composite curve evoked by cortisone reflects a depression of lipogenesis associated with a decreased R Q Actual R Q measurements have indeed shown that cortisone added *in vitro* lowers the R Q and acetate uptake of mammary tissue slices from lactating rats thus providing additional and less equivocal evidence that cortisone inhibits lipo genesis from small molecules (Balmain, Folley and Glascock, 1952 *a*) It may be noted in passing that by contrast with the situation regarding mammary tissue taken from rats just before or after parturition changes in the slope of the composite respiration curve of *fully lactating* tissue may with fair confidence be regarded as reflecting changes in R Q which in turn are related to changes in the rate of lipo genesis

The most conclusive evidence of the inhibitory effect of cortisone on lipogenesis in lactating mammary gland slices has come from studies with labelled glucose and acetate Studying mammary gland slices from rats in early lactation (days 2–4) we have observed that cortisone *in vitro* markedly inhibits the incorporation of C^{14} into the fatty acids of slices incubated in glucose × [*carboxy* C^{14}] acetate It should be noted that it does this without appreciably affecting the composite respiration curve at this stage (Balmain and Folley, 1952) Slices from rats in full lactation (days 12–15) have been incubated with C^{14} glucose + tritio-acetate as substrates and we have found that cortisone significantly decreases the incorporation of both isotopes into the fatty acids of the slices Insulin as stated above potentiates lipogenesis in *lactating*

of chorionic gonadotrophin is increased by ACTH pretreatment and decreased after adrenalectomy

Highly purified ACTH preparations in our experience never showed gonado trophic stimulating or inhibiting activity It is very interesting that stress can cause gonadal hypertrophy However it must not be overlooked that thyroid activity also can be considerably increased by stress

D J Ingle The same stimulus does not always produce the same response as there are many conditioning factors involved I do know in contradiction of Professor Zuckerman that prolonged severe stress can cause atrophy of the gonads presumably due to suppression of gonadotrophin

C H Li In 1936 Davidson and Moon (*Proc Soc Exp Biol Med* 35 281) found that a pituitary extract rich in ACTH and prolactin caused enlargement of the seminal vesicles and prostates of castrated rats We have failed to confirm this during the last ten years experimenting with purified ACTH preparations We also tried in jecting the animals with ACTH administered along with other hormones (luteinizing hormone follicle stimulating hormone prolactin etc) still with negative results I am wondering whether the observations of Davidson and Moon were possibly due to another factor which has not yet been elucidated

Therefore I feel that there is no evidence at the present time to indicate that ACTH stimulates the suprarenals to produce a hormone which has an effect on the accessory reproductive organs

F T G Prunty It is true that without the biologists the chemists do not know what to look for, but the process of disarticulation is of great importance It has to be remembered that compounds may have a synergic action However the chemists are limited by their methods and must work on isolated compounds They must be guided by the findings of biologists when selecting compounds on which to work

A lot of work has been done on the adrenal ascorbic acid depletion factor and yet we do not really know whether it exists in the intact animal or what significance it has ACTH should for the present be defined very generally

With regard to the experiments of Dr Clayton and myself quoted by Professor Zuckerman—mouse granulation tissue is an excellent test object for ACTH There was no inhibition of the granulation tissue by ACTH in adrenalectomized animals unless they were at the beginning of the breeding season pregnant or pre treated with chorionic gonadotrophin This is in agreement with Hill s work on the survival of adrenalectomized animals with an active ovary Progesterone had a slight effect similar to that of ACTH but had to be given in very large doses We suggest that the gonads are capable of responding to ACTH under the influence of luteinizing hormone

ACTH promotes the inhibiting effect by the ovary more than can be accounted for by the progesterone secreted Dr Hoagland s slide suggests how progesterone may by metabolized to corticoids

F G Young As I said yesterday we have not yet obtained a preparation of the adrenal weight increasing factor which fails to stimulate the gonads of the hypophysectomized rat when administered in large doses

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Discussion

ON PAPERS BY (1) ZUCKERMAN, (2) FOLLEY

Chairman Dwight J Ingle

M Reiss We have lately shown, in collaboration with Brimblecombe and Halkerston that pretreatment of immature female rats with ACTH suppresses to a high degree the follicle stimulating action of the gonadotropic hormone contained in pregnant mare s serum This action is decreased after adrenalectomy The luteinizing activity

respect to the adrenal cortex, I shall hesitate to assume as more than a possibility the idea that the gonads normally secrete corticoids

S Zuckerman Dr Reiss s experiments in which I understand he used preparations of ACTH free of demonstrable gonadotrophin, enlarge the area of interaction which I have discussed and—so far as I can make out—support my general conclusions So too, do the observations made by Dr Prunty, as well as Professor Young s reminder that his purest preparations of the adrenal weight increasing factor are also to some extent gonadotrophic Dr Li s remarks are of great interest but even if he interprets his own experiments as implying that ACTH cannot stimulate the adrenals to produce a steroid which acts on the accessory reproductive organs and even if he believes that Davidson and Moon s results were due to some factor other than ACTH the fact remains, as I have already pointed out that these workers, as well as Nelson observed change in gonadectomized animals which can only be interpreted as showing that some tissue presumably the adrenal cortex, can in the absence of the gonads and in response to some pituitary hormone secrete gonadal hormone

With Dr Ingle I agree completely My observations in no way conflict with his statement What I have been concerned to show was that in certain conditions which I have defined stress may be associated with hypertrophy and not involution of the gonads Moreover I was careful to emphasize that under normal conditions the adrenal glands and the gonads however interrelated they may be, have quite separate endocrine functions

This brings me to Professor Young s main remarks The essential point I tried to make in the first part of my paper was that generally speaking biochemistry is not a predictive science in the sense that it allows us to forecast the hormonal action of some unknown compound derived from the pituitary from a knowledge of its *molecular configuration Such being the case so I argued the essential criteria* by which we define the specific nature of so called pure hormones must be based on a knowledge of biological function I was careful to draw the bulk of the evidence with which I supported this thesis and that of the interaction of the hormonal activities of the adrenal cortex and gonads from experiments the significance of whose results did not depend on the purity or otherwise of preparations of pituitary hormones For example it is largely irrelevant whether the experiments of Nelson and of Moon and Davidson to which Dr Li has referred were carried out with pure ACTH or not Given that their findings are reproducible what is relevant is the fact that changes were induced by pituitary preparations in the accessory reproductive organs of animals whose gonads had been removed

Dr Nelson has adduced strong evidence in favour of the view that the adrenal cortex can produce androgen But I disagree with Professor Young if he really believes that this evidence is different in terms of scientific method from that which Woolley has provided Both workers assume that it is an androgen which is produced because the hormone stimulates the male accessories of experimental animals The fact that Dr Nelson knows exactly what the androgen is from the chemical point of view is interesting But it is of no consequence to the diagnosis of the molecule s androgenic properties unless Professor Young really believes that one can overlook the fact that such a diagnosis either depended on biological experiments carried out by Dr Nelson or by some other worker who established the biological

With reference to Dr Nelson's observations, I believe I am right in saying that he not only isolated a substance from adrenal vein blood which he showed chemically to be a steroid, but also demonstrated in suitable tests that the isolated substance was androgenic It seems to me of great importance that in the blood flowing from the adrenal glands of a *normal* animal (I assume that the cow on which Dr Nelson operated may be regarded as normal) there has been shown to be present an androgenic steroid To me this is much more convincing evidence that the adrenal gland can secrete androgens under normal conditions than the biological arguments put forward by Professor Zuckerman, interesting and suggestive though they be I was not previously aware of the experiments of Woolley which Professor Zuckerman quotes in this connection, but however interesting such biological arguments may be I still prefer Dr Nelson s direct and biochemical approach to the problem

I was interested in Professor Zuckerman's general ideas about the relationship of chemistry and biology but regard them as rather dangerous The biologist sometimes uses preparations of hormones of biological origin provided by the biochemist as though they were pure chemical substances to be used as ordinary chemical reagents One surely should regard as doubtful or at least as provisional the interpretation which the biologist provides for the results of his experiments until such time as the substances themselves have been clearly characterized chemically and preferably synthesized in the laboratory, and biological activity obtained with the artificially synthesized material This is particularly true for the so called protein hormones of the pituitary gland

At present I do not try to assess the physiological significance of the ascorbic acid *reducing pituitary substance and I do not regard the effect obtained with it as more* than a suitable one for the control of the isolation of a chemical fragment from the anterior pituitary gland When ultimately enough of such separated chemical entities—such disarticulated fragments—have been obtained and their chemical natures determined and once we have analysed in biochemical terms the metabolic activity of the anterior pituitary lobe both in general and with particular respect to the production of these separable entities and the control of their production, then and only then shall we be able to rearticulate our chemical and biological fragments into a pattern of general physiological significance At present we can make guesses about the nature of parts of the pattern but until we have more chemistry and biochemistry we are liable to be dangerously misled

I agree with Professor Zuckerman that for some time the evidence has been strong that the adrenal cortex can secrete androgens but to me the evidence has not been convincing that such secretion takes place in the normal animal *But I do accept the* results of Dr Nelson s direct and biochemical approach to the problem Until evidence of this kind has been provided with respect to the production of cortisone or other corticoids by the gonads I shall continue to hesitate to assume that such a *production normally occurs I believe that the gonads may be able to secrete small* amounts of many hormones since I prefer to think of hormones as metabolites—the products of cell metabolism in a general way There is good evidence that thyroxine can be produced in small amounts in tissues other than the thyroid gland and it may well be that all cells provide all hormones in minute quantities But in the absence of direct experimental evidence of the kind provided by Dr Nelson with

respect to the adrenal cortex I shall hesitate to assume as more than a possibility the idea that the gonads normally secrete corticoids

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properties of the compound with which Dr Nelson identified the one he found To my way of thinking too, there is nothing essentially different in the mental processes which led Woolley on the one hand, and Nelson on the other, to the belief that the androgen was actually produced in the adrenal cortex, and not in some other tissue Dr Nelson reached this conclusion because he obtained the material he tested from the adrenal vein, and Woolley from a very extensive set of biological experiments on gonadectomized animals which allowed of no other conclusion

If all this were otherwise I would happily follow Professor Young in his optimism that the physiological pattern we are discussing will only be properly discerned when we have more laboratory made chemical fragments of the anterior pituitary in front of us His is not a dangerous idea—its main characteristic is simplicity Clearly we shall all be richer in knowledge if more recent developments in the chemistry of the pituitary hormones can help give the pattern we know better shape—especially if at the same time they succeed in transforming endocrine chemistry into what I have called a predictive science But at present it is salutary to recall that the essential outlines of our knowledge of pituitary function were established long before the stage of the pure preparations we are talking about

P Fourman I am surprised Dr Folley that DCA should be more effective than cortisone in maintaining lactation in adrenalectomized rats, could this be attributed to the similarity between DCA and progesterone? What is the effect of progesterone? Can you throw any light on the occasional breast development in Addisonian patients under treatment with DCA or cortical extract?

S J Folley Progesterone, in the rat at any rate has no inhibitory effect on lactation in doses as high as 15 mg daily In our earlier experiments DCA proved more effective in maintaining lactation after adrenalectomy in our rats than such doses of cortisone as we were able to give at the time Since cortisone has become more readily available we have shown that it is about as effective as DCA when given in equal dosage but neither hormone alone completely restores lactation in our adrenal ectomized rats

Breast development in Addisonian patients receiving DCA or cortical extract is intelligible in the light of our finding some years ago that DCA exhibits mammo genic activity in the mouse

Some Observations on the Urinary Adrenocortical Steroids

by

G F MARRIAN

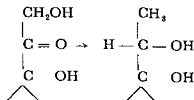
Department of Biochemistry University of Edinburgh

21 DEOXYSTEROIDS AS METABOLIC PRODUCTS OF THE C_{21} ADRENOCORTICAL HORMONES

APART from the 17 ketosteroids interest in the urinary steroids of adrenocortical origin has hitherto been largely confined to the C_{21} 21 hydroxy compounds and both biological and chemical methods for their quantitative determination in urinary extracts have been widely used for the assessment of adrenocortical activity in man However there are reasons for supposing that C_{21} 21 deoxysteroids (i e 20 methyl steroids) may not be unimportant metabolites of the adrenocortical hormones and it seems possible that the determination of the urinary excretion of such steroids might provide a useful additional means of assessing adrenocortical activity

A number of different C_{21} 20 methyl steroids have been isolated by various workers from the urine of human subjects with adrenocortical hyperactivity (see Table I) and although such urinary steroids may arise in part from 20 methyl steroids secreted by the adrenal cortex there is some reason to believe that they might also be formed by metabolic reduction of the primary hydroxyl group of 21 hydroxy steroids

Thus Cuyler Ashley and Hamblen (1940) and Horwitt Dorfman Shipley and Fish (1944) *isolated pregnane 3α 20α diol from the urine of human subjects after* the administration of 11 deoxycorticosterone acetate Mason (1948) isolated pregnane 3α 20α diol 11 one after the administration of 11 dehydrocorticosterone while more recently pregnane 3α 17α diol 11 20 dione has been isolated after the administration of cortisone acetate by Lieberman Hariton Stokem Studer and Dobriner (1951) It seems possible therefore that reduction of the 21 hydroxyl group of the adrenocortical hormones to methyl may be a general metabolic pathway of these hormones in the body and if this is indeed the case the three 17 hydroxy adrenocortical hormones might all be expected to yield metabolic end products with 17α 20α dihydroxy 20 methyl side chains thus



A method for the quantitative determination of such 17 20 dihydroxy 20 methyl steroids in alkali washed chloroform extracts of urine has recently been elaborated by Cox (1952) This method involves oxidation with periodic acid and determination of acetaldehyde by the colorimetric method of Barker and Summerson (1941), and it permits of the simultaneous determination of formaldehyde formed from formal dehydogenic substances present The accuracy of the method has been tested by experiments in which pure pregnane 3α 17α 20α triol was added to alkali washed extracts of urine from normal men The problem of hydrolysing quantitatively with out loss the conjugated forms of the urinary 17 20 dehydroxy 20 methyl steroids has yet to be solved, and accordingly it cannot be claimed that the procedure permits of the quantitative determination of these steroids in urine In this respect the method is probably neither better nor worse than the chemical and biological methods in general use for the determination of the 21 hydroxy adrenocortical steroids in urine

In the course of this work Cox detected the presence of small but not insignificant amounts of acetaldehydogenic material in control extracts of urine to which no pregnane 3α 17α 20α triol had been added and an attempt to isolate this material is at present in progress This work is only at a preliminary stage but a small amount of a crystalline acetaldehydogenic substance has been isolated from β glucuronidase hydrolysed urine of normal men and the chromatographic behaviour of this sub stance suggests that it may be pregnane 3α 17α 20α triol

HYDROLYSIS OF THE CONJUGATED ADRENOCORTICAL STEROIDS IN URINE

Undoubtedly the biggest unsolved problem in connection with the quantitative determination in urine of formaldehydogenic reducing or glycogenic steroids is that of hydrolysing the conjugated forms which are present in urine completely and with out loss prior to their extraction Although the 17 deoxy adrenocortical steroids are not rapidly destroyed by treatment with hot dilute acid, the 17 hydroxy ones are accordingly boiling the urine after acidification cannot be employed as a hydrolytic procedure However it is well established that the yields of extractable corticoid, determined as formaldehydogenic, reducing or glycogenic material, can be signifi cantly increased—usually about two fold—if the urine is acidified to pH 1–2 and either extracted immediately or extracted after standing at room temperature for twenty four hours Both of these methods of mild acid pretreatment have been widely employed in the quantitative determination of the urinary adrenocortical steroids but they are open to certain serious objections

Firstly it seems clear that the increased yield of extractable reducing or formalde hydogenic material which results when urine is extracted immediately after acidifi cation cannot be due to the hydrolysis of conjugates since the effect of acidification can be reversed by neutralization before extraction (Heard Sobel and Venning 1946 Paterson Cox and Marrian 1950) Rather this increase would seem to be due to the extraction in the unionized form of some type of unhydrolysed conjugate which is not a glucuronide (Paterson and Marrian 1951)

Secondly, as shown by Paterson Cox and Marrian (1950) allowing the urine to stand at pH 1 for several hours at room temperature may result in considerable loss of extractable formaldehydogenic material as determined by periodate oxidation

This loss is not seen in all urine specimens and recent work suggests that it may be due to causes other than an inherent acid lability of any of the formaldehydogenic steroids in urine However, it seems clear that whatever may be the true explanation of the apparent destruction of extractable formaldehydogenic material, this is potentially a considerable source of error in determinations in which acidified urine is allowed to stand for several hours prior to extraction

Thirdly the work of Kinsella Doisy and Glick (1950) Cox and Marrian (1951), Venning (1951) and Cohen (1951) has shown that the yields of extractable reducing formaldehydogenic or glycogenic material which can be obtained from urine after incubation with preparations of β glucuronidase are significantly greater than those which can be obtained after mild acid pretreatment

The above mentioned work on the enzymic hydrolysis of the conjugated reducing formaldehydogenic and glycogenic material in urine is of considerable importance in so far as it has focused attention upon the deficiencies of the widely employed methods of acid pretreatment On the other hand it would be dangerous to assume that it has yet provided a satisfactory method of hydrolysing the conjugated urinary corticoids which is suitable for routine quantitative work Thus the possibility that urine may contain variable amounts of β glucuronidase inhibitors is by no means a remote one and furthermore the work of Paterson and Marrian (1951) has shown that urine may contain appreciable amounts of conjugated formaldehydogenic material which is not hydrolysed by β glucuronidase preparations

It may be remarked that in the published work on the enzymic hydrolysis of the conjugated urinary corticoids there is little evidence to justify the claim that the effective hydrolytic agent in the relatively crude enzyme preparations employed is indeed β glucuronidase It has now been shown (Cox and Marrian unpublished) that the liberation of both free formaldehydogenic and free acetaldehydogenic material from butanolic extracts of urine on incubation with ox spleen preparations is largely inhibited in the presence of saccharate Since saccharate is a powerful inhibitor of β glucuronidase (Karunairatnam and Levvy 1949) this finding provides a measure of support for the belief that the hydrolysis of the conjugates is effected by this enzyme

TABLE I

20 methyl steroids isolated from the urine of human subjects with adrenocortical hyperactivity

Steroid	Condition of subject	Authors
Pregnane 3α 17α 20α triol	Adrenocortical hyperplasia	Butler and Marrian (1937 1938)
Pregnane 3α 17α 20α triol	Adrenocortical hyperplasia Adrenocortical tumour	Mason and Kepler (1945)

TABLE I—*continued*

Steroid	Condition of subject	Authors
Pregnane 3α 17α diol 20 one	Adrenocortical hyperplasia Adrenocortical tumour	Lieberman and Dobriner (1945)
Pregnane 3α 17α diol 20 one	Adrenocortical hyperplasia	Miller and Dorfman (1950)
Pregn 5 ene 3β 17α 20α triol	Adrenocortical tumour	Hirschmann and Hirschmann (1900)
Pregn 5 ene 3β 17α diol 20 one	Adrenocortical tumour	Hirschmann and Hirschmann (4947)
Pregnane 3α 20α diol 11 one	Adrenocortical hyperplasia	Lieberman *et al* (1950)
Pregnan 3α ol 11 20 dione	Adrenocortical hyperplasia	Lieberman *et al* (1950)
Pregnane 3α 17α diol 11 20 dione	Treated with ACTH	Lieberman *et al* (1951)

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HIRSCHMANN H, and HIRSCHMANN F B (1950) Steroid excretion in a case of adrenocortical carcinoma V Δ^5 pregnenetriol 3β 17α 20α *J biol Chem* 187 137

HORWITT B N DORFMAN R I SHIPLEY R A, and FISH, W R (1944) Metabolism of the steroid hormones IV Conversion of desoxycorticosterone to pregnane diol 3 (α) 20 (α) in man and in the chimpanzee *J biol Chem* 155 213

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Discussion

Chairman F G Young

A Puck I should like to report briefly some preliminary results obtained during an investigation into adreno cortical function in cases of carcinoma of the uterus

On the one hand it seemed possible that in malignant disease the organism might be subjected to stress in response to which hyperfunction of the adrenal cortex might

occur On the other hand the fact that sex hormones not infrequently have a beneficial effect in uterine carcinoma appeared to suggest the reverse possibility of hypo function or perhaps dysfunction of the cortex, so that the administration of hormones would be a form of substitution therapy We therefore have investigated 17 ketosteroid excretion—as an index of cortical function—in a series of cases, using a modified Zimmermann Callow technique and making final colorimetric measurements with the Pulfrich Stufen photometer

In a control series of healthy women, of the same age range as our carcinoma cases, we found an average excretion of 9 mg per day varying between 7 to 12 mg The results of many other investigators, using a variety of methods, give figures ranging from 4–16 mg per day Our own data on 17 ketosteroid excretion are based on 135 separate determinations in twenty cases of carcinoma of the uterus It will be seen that in most of these cases the keto steroid output is reduced sometimes very markedly As compared with an average daily excretion in healthy women of 9 mg per day, the patients with cancer of the uterus excreted an average of 4 8 mg per day

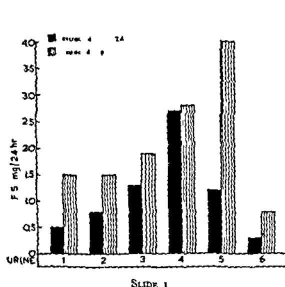
These figures show a greater fall than has been reported in other types of malignant disease Thus Dobriner *et al* found in a variety of malignant diseases (cancer of larynx, prostate stomach, and lymphatic leukaemia) an average reduction of about 20 per cent as compared with normal controls Raymond in Switzerland found only a slight decrease in different types of malignant disease, not including carcinoma of the uterus Our figures then fit in better with the hypothesis of diminished adreno-cortical function rather than with that of a stress response associated with hyper function

R I S Bayliss It is clear from Professor Marrian s remarks that our lack of knowledge as to the best methods of hydrolysing the various conjugated steroids in urine does not inspire confidence in the results obtained by any of the procedures now used for estimating the urinary corticosteroids In an attempt to evolve a practical technique for hydrolysing and extracting the so called free steroids and their conjugates which so far have been recognized as being present in urine a number of experiments have been carried out using a modified Daughaday formaldehydogenic method (Bayliss 1952)

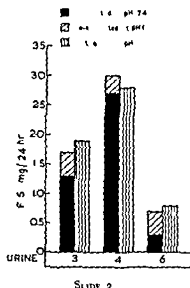
Slide 1 shows that urine extracted immediately after acidifications to pH 1 0 usually yields a greater amount of formaldehydogenic substance than urine extracted at pH 7 4 It is said that at neutral pH free steroids are extracted In practice it is difficult to collect a twenty four hour specimen of urine free from bacterial contamination Bacteria may hydrolyse glucuronide conjugates and the amount of 'free steroid obtained may be influenced by the extent and type of bacterial contamination

The greater yield at pH 1 0 may be due to the rapid hydrolysis of a conjugated compound, and slide 2 shows that there is no substantial advantage in first extracting urine at neutral pH and then re extracting it at pH 1 0 The yield from the double extraction procedure does not differ significantly from the value obtained from a single extraction immediately after acidification This must mean that there is no appreciable destruction of free steroid present in neutral urine when the urine is acidified to pH 1 0 and extracted immediately

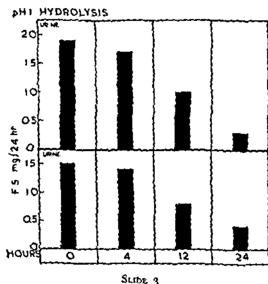
Prolonged acidification at pH 1 0 may decrease the yield of formaldehydogenic substances (slide 3) This sort of result is not always obtained Paterson *et al* (1950) *found that* although the yield after four hours' acidification decreased, it rose again



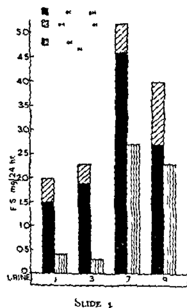
Slide 1



Slide 2



Slide 3



Slide 4

in twenty four hours suggesting an initial destruction of steroid followed later by the slow hydrolysis of a conjugated compound Slide 4 shows that the amount of destruction and the yield by slow hydrolysis varies from one urine to another In urine 1

and 3 it would seem that most of the free steroids and those liberated from the conjugate rapidly hydrolysed at pH 1 0 were acid labile and destroyed by acidification for twenty four hours On the other hand in urines 7 and 9 there was less destruction of the free substances because the values obtained after acidification to pH 1 0 for twenty four hours were greater than those obtained when the urine was extracted for the second time

None of these procedures causes appreciable hydrolysis of glucuronide conjugates After urine has been acidified and extracted immediately, and then left for twenty four hours and extracted again, considerable amounts of formaldehydogenic substances can be obtained by hydrolysis with β glucuronidase The yield after enzymic hydrolysis is often large but the amount of glucuronide conjugates excreted varies widely from one day to another Until the reason for these apparent fluctuations is forthcoming the routine use of enzymic hydrolysis alone is not satisfactory

G F Marrian I should like to ask Dr Bayliss whether he washed the chloroform extracts

R I S Bayliss Yes

G F Marrian I entirely agree with Dr Bayliss that the destruction of formaldehydogenic steroid in acidified urine is variable and is not always observed It is clear that we are here dealing with a phenomenon the nature of which we do not understand

C L Cope I have been interested in the extraction problem but from a rather different angle I took the view that the output of biologically active steroid in the urine was likely *to reflect the concentration of such steroid prevailing in the internal* environment Among the inert steroid metabolites which form at least 90 per cent of the total may well be some which have never had any biological activity With Miss Hurlock I have studied the effect of hydrolysis with β glucuronidase on the yield of biologically active hormone which is presumably composed of cortisone and Compound F

We have been examining a series of human urines for their content of biologically active hormones as tested by their production of eosinopenia in adrenalectomized mice We compared the level of activity of the extract obtained by a single extraction after twenty four hours hydrolysis at a pH of 1 0 with that obtained by a triple extraction procedure consisting of forty eight hours glucuronidase treatment followed by adjusting the pH to 1 0 and finally hydrolysing at this pH for twenty four hours extracting with chloroform at each successive step and finally pooling the resultant extracts as advocated by Bayliss (1952) *As control for the possible presence of active steroid in the glucuronidase the urine from a case of Addison s disease* was used and was found to give a negative result The triple extraction procedure using glucuronidase approximately doubled the yield, as compared with the single extraction method after pH 1 0 hydrolysis at all levels of steroid output from normal up to around 1 mgm daily

The increased yield of biological activity varied from 20–150 per cent We never got a ten to twenty fold increase such as has been reported by some workers for formaldehydogenic steroid

L R Broster This work is of the greatest importance to the clinician and has revolutionized one aspect of surgery

The urine analysis of the ketosteroids is of great assistance in differential diagnosis For instance it is the only certain way that adrenal and pituitary Cushing s syndrome can be differentiated It is essential too in assessing cases of sexual precocity and intersexuality The urinary ketosteroids have become an important yardstick in diagnostic surgery

G A Overbeek Professor Marrian a vast amount of data has been published in the past on the amounts of corticosteroids in the urine In view of your results what must we now think of these figures?

G F Marrian I believe the value of much of these data is questionable

F G Young Dr Puck, have you examined the urinary steroids in any cases of carcinoma of the uterus with diabetes?

A Puck No

M Finkelstein While I do not think that anything could be added at present to what Professor Marrian has said about the problems of hydrolysis I would like to make a comment in connection with the extraction and estimation of urinary steroids

In the early thirties Professor Marrian observed that oestrogens develop fluorescence on being heated with sulphuric acid A few years ago we described a fluorometric method for quantitative determination of urinary oestrogens using phosphoric acid for development of the fluorescence Recently we have undertaken an investigation of both the phenolic and neutral urinary fractions using this method We have observed that the neutral water soluble fraction also exhibits a relatively strong fluorescence on being heated with phosphoric acid This phenomenon has been shown by all samples of urine tested by us so far The urinary excretion of this fluorogenic substance was about 700γ–1200γ/litre in the human female and about 1500γ/litre in the male expressed for reasons of convenience in terms of 17 hydroxy corticosterone During this investigation we came across two cases of female pseudo hermaphrodites in which the excretion was about 4000γ–5000γ/litre urine We performed a large scale fractionation of urine of these two patients and we have asked Professor Reichstein to undertake an investigation of the neutral water soluble fraction with a view to isolation of crystalline substances Professor Reichstein isolated several crystalline compounds out of this fraction and one of them showed the characteristic fluorescence These substances are apparently different from the steroids hitherto isolated from urine If these compounds prove to be steroids it seems that they escape detection in the current methods of urinary steroid analysis This is not surprising since after all these methods determine specific groups in the steroid molecule These groups are not necessarily present in every urinary steroid and there may be many other steroids present which have not yet been identified

The Rôle of the Adrenal Glands in Infection and Intoxication

by

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It has long been suspected that the adrenal glands play an important role in the defence mechanism during infection and intoxication This opinion has been based upon a number of observations, including the fact that removal of the adrenal glands results in a profound decrease in the ability of animals to survive infection or survive exposure to noxious stimuli in general (Marmorston Gottesman and Perla 1931 Marmorston Gottesman and Perla 1932 Marmorston Gottesman, Perla and Vorzimer 1939) Moreover the adrenal glands are known to undergo a number of morphologic and chemical changes as a result of infection or intoxication (Goldzieher 1929) These vary from a mild non specific cortical hypertrophy to severe destructive lesions characterized by oedema haemorrhage and necrosis Loss of lipid and ascorbic acid frequently accompany these morphologic changes

In view of the foregoing observations many investigators were of the opinion that adrenal cortical extract (ACE) might find therapeutic use in the treatment of human infectious diseases Few studies of this nature have been made, but favourable effects following the injection of cortical extract have been observed particularly in ameliorating the exhaustion and muscular asthenia (Wenner and Cone 1934) Marmorston and Perla have reported beneficial effects with cortical extracts in humans for the treatment of influenza upper respiratory infections and pneumonia The effects observed were a definite increase in the sense of well being stimulation of the appetite and shortening of the convalescent period (Perla and Marmorston Gottesman 1941) However large amounts of ACE had no apparent effect on the survival rates of animals infected with a variety of pathogenic micro organisms It should be noted however that in most cases the ACE was tried in acute overwhelming infections and that under such circumstances minor alterations in the defence mechanism would be difficult to detect

Except for certain special studies the adrenal cortical hormones attracted little further attention until the epoch making discovery of Hench and Kendall in 1949 concerning the effects of cortisone in rheumatoid arthritis This discovery stimulated research in practically all fields of medicine and interest in the problem concerning the role of adrenal cortical hormones in infection and intoxication was again revived In view of the potential use of adrenocorticotropic hormone (ACTH) and cortisone in patients for the treatment of rheumatoid arthritis the effects of these potent physiologic agents on infection acquired new and added significance

A number of experimental and clinical reports have appeared describing the effects of ACTH and cortisone in various infectious diseases including certain experimental bacterial, viral rickettsial protozoan, and fungus diseases

One of the first clinical papers to appear was that of Kass, Ingbar, and Finland (1950) describing the effects of ACTH in pneumonia These authors reported that patients with viral and pneumococcal pneumonia experienced a sense of well being and a sharp drop in temperature within twenty four hours after the injection of ACTH However, the dramatic improvement in the signs and symptoms of the patient was not accompanied by a corresponding improvement in the infectious state since the patients suffering from pneumococcal pneumonia continued to have a bacteraemia In rats and rabbits infected with *Diplococcus pneumoniae,* the survival rate was definitely less favourable when large doses of cortisone or ACTH were administered (Robinson, 1951, Glaser *et al*, 1950, Glaser *et al*, 1951, Robinson and Smith to be published) Essentially the same deleterious effects have been reported in pneumonia and other infections in rats and rabbits due to *Streptococcus hemolyticus* (Mogabgab and Thomas 1950, Mogabgab and Thomas 1950, Hahn *et al*, 1951)

Studies on experimental tuberculosis in the mouse, rat, rabbit, and guinea pig (D Arcy Hart and Rees 1950 Michael, Cummings and Bloom, 1950, Spain, 1951 Lurie *et al*, 1951, Cummings and Hudgins 1951, Ebert, 1951, Karlson and Gainer, 1951, LaMaistre and Tompsett 1951) show that cortisone and ACTH tend to enhance the disease and increase the mortality from the infection Because of the deleterious action of these hormones in experimental tuberculosis, and the evidence along similar lines observed in man (Leading article, *Lancet*, 1950 Popp, Ottosen and Brasher, 1951 Committee on Medical Research of the American Trudeau Society 1951 Kleinschmidt and Johnston 1951, Fred *et al* 1951), it has been recommended (Committee on Medical Research of the American Trudeau Society 1951), that these hormones not be used in patients with tuberculosis until such time as further investigation has shown such administration to be safe It is important to remember, however that very little is known about the effects of small doses of cortisone or ACTH on experimental or human tuberculosis In this connection, the recent work of Lurie (1951) is of particular interest This author reported that tuberculosis in rabbits is accompanied by marked hypertrophy of the adrenal cortex Furthermore the degree of hypertrophy of the adrenal cortex of natively resistant rabbits is much greater than that of susceptible rabbits similarly infected Lurie, like others noted however, that doses of cortisone which produce intense physiologic effects in rabbits enhanced the number of tubercles resulting from the inhalation of human tubercle bacilli On the other hand, the spread of the disease to the tracheo bronchial lymph nodes and also to other organs, was markedly reduced by cortisone and this author concluded that cortisone can transform a rabbit of high genetic susceptibility to tuberculosis into one that shares some of the essential pathological responses characteristic of natively resistant rabbits

Relatively little has been published regarding the effect of cortical hormones in syphilis, but the few reports that have appeared are extremely interesting Turner and Hollander (1950) found that under the influence of cortisone, syphilomas induced in rabbits by intradermal or intratesticular inoculation of *Treponema pallidum*

show striking changes consisting of a conversion of the firm lesion into a soft, spongy mass There was excessive accumulation of a mucoid material, tentatively identified as hyaluronic acid The treponemes were unusually abundant and the production of Wassermann reagin seemed to be inhibited In nine cases of interstitial keratitis due to congenital syphilis cortisone acetate applied locally as eye drops in conjunction with procaine penicillin in oil given intramuscularly, appeared to exert a beneficial effect within twenty four hours in all but one case (Simpson *et al* 1951)

A number of reports have also appeared concerning the effects of ACTH and cortisone in various viral infections in animals and humans Some of the virus infections studied to date include Japanese B encephalitis in mice (Vollmer and Hurlbut 1951) poliomyelitis in mice (Shwartzman 1951) humans (Cornell *et al*, 1950) experimental herpes simplex keratitis in rabbits (Thygeson, Geller and Schwartz 1951 Hallett *et al*, 1951), Coxsackie virus in mice (Kilbourne and Horsfall 1951) and the Lee PR8 and mumps virus in eggs (Kilbourne and Horsfall 1951) In general these hormones tend to increase the titre of viruses in tissues of infected animals In man cortisone has generally failed to produce improvement in ocular infections of viral origin and may in some cases be deleterious (Thygeson 1951) A study in human poliomyelitis involving seventy cases revealed that cortisone had no beneficial or adverse effect on the disease as judged by the usual criteria for measuring the severity of this disease (Cornell *et al* 1950)

Other isolated reports concerning these hormones and infection include the effects of ACTH or cortisone on typhoid fever (Woodward *et al* 1951, Smadel, Ley and Diercks 1951) coccidiosis immitis infection in rats (Cavallero and Sala 1951) trypanosomiasis in rhesus monkeys (Wolf *et al* 1951) brucellosis (Abernathy 1951) trichinosis (Luongo Reid and Weiss 1951, Rothenberg 1951) and malaria (Schmidt and Squires, 1951)

To date cortisone and ACTH have been given to thousands of patients and except for tuberculosis and certain infections of bacterial and viral origin involving the eye the incidence of serious complications resulting from intercurrent infection in man has been surprisingly low The reasons for the contrasting results in animals and man are not known at present and hence the ultimate role of cortisone in the treatment of infectious disease of humans must await further study

The deleterious effects of large doses of ACTH and cortisone on the resistance of animals to certain infectious diseases offers an excellent device for gaining further insight into the factors influencing the process of infection and host resistance In addition knowledge acquired in such studies might aid in elucidating the mechanism of action of these hormones in other diseases Therefore the present study was initiated to determine the effect of ACTH and cortisone on a standardized infectious process using a pneumococcal cellulitis in rabbits as a working model This experimental disease permitted examination of a number of defence reactions important to the survival or death of the infected host

MATERIALS AND METHODS

Details of the procedures employed in the studies herein described have been presented elsewhere (Robinson and Smith to be published)

Infection was produced by injecting 0 1 cc of a diluted virulent Type I pneumo coccal culture intradermally into the shaved abdomen of white male rabbits The culture was grown for six hours at 37° C in brain heart infusion broth containing 5 per cent defibrinated blood Serial ten fold dilutions of the culture were made in broth through 10 ³ immediately before animal inoculation This quantity of culture contained approximately 250 000 to 300,000 pneumococci determined by plating the cultures in blood agar Various laboratory tests were performed according to standard methods as described elsewhere (Robinson and Smith, to be published)

RESULTS

Effect of large multiple doses of cortisone and ACTH In these experiments sufficient cortisone and ACTH were given to produce intense physiologic effects on the carbo hydrate, fat, and protein metabolism This was evidenced by the hyperglycaemia lipaemia, and increased excretion of nitrogen In addition the adrenal glands and lymphoid tissue were atrophied and the liver markedly enlarged as a result of the deposition of fat and glycogen Cortisone treatment was initiated five days before the bacterial injection and consisted of daily subcutaneous injections of 10 mg of cortisone per rabbit In the ACTH studies a range of doses from 0 1 to 1 0 mg was employed This substance was administered subcutaneously every hour night and day in order to maintain maximal physiological effects Treatment began two and a half days before infection was initiated and continued for three days after the bac terial injection

Survival of infected animals Almost all of the control animals survived the infection over the ten day observation period On the other hand only 20 per cent of the cortisone treated animals lived and none of the animals receiving ACTH survived (Figure 1) In both treated groups deaths began to occur within forty eight hours after the pneumococcal injection Studies with hydrocortisone produced the same results as those described for cortisone Control toxicity experiments with ACTH and cortisone showed that the hormones were not lethal to non infected rabbits when given in the manner described above

Skin lesions The gross appearance of the skin lesions in the control and treated animals was markedly different In the untreated rabbits the lesion developed slowly over a forty eight hour period and at this time was characterized by the formation of an elevated oedematous pouch usually confined to an area of 40–50 sq cm (Figure 2) Thereafter the lesion remained essentially the same for the next four to five days and then gradually subsided over a fifteen to twenty day period In the cortisone or hydrocortisone treated rabbits the skin lesion developed much more rapidly Within forty eight hours it attained an area of approximately 120 sq cm and had the appearance of a diffuse, thickened somewhat elevated erythematous plaque The skin lesions in the ACTH treated animals in contrast to those in either the control or the cortisone treated animals, made their appearance slowly and were very small flat areas approximately 1–2 sq cm in diameter There was little or no erythema surrounding the injection site

Microscopic examination of the skin lesion in the various groups of animals revealed marked differences In the case of the control animals there was an early

infiltration of polymorphonuclear leucocytes These cells made their first appearance within five to ten minutes following the bacterial injection Within twelve hours there were large numbers of polymorphonuclear leucocytes present many of which were already undergoing necrosis (Figure 3) Numerous phagocytized pneumococci were noted and there was a moderate amount of oedema fluid distributed throughout the area In the cortisone treated animal there was a marked delay in the influx of polymorphonuclear leucocytes In fact, the early lesions in these animals were relatively acellular areas containing considerable oedema fluid and many unphago cytized pneumococci (Figure 4) However eighteen to twenty four hours after the injection large numbers of polymorphonuclear leucocytes and some macrophages were also found in the skin lesions of the treated animals and many pneumococci were seen within the white cells At this time it was difficult to distinguish micro scopically between the lesion in the control and the cortisone or hydrocortisone treated groups

The lesions in the ACTH treated animals were essentially free of oedema fluid and as in the cortisone treated animals the cellular components were essentially absent However there were large numbers of pneumococci seen lying free in the intercellular spaces

Blood culture studies The untreated rabbits developed a transient bacteraemia of low magnitude which had its onset approximately four hours after the intradermal injection of the pneumococci and reached a peak within a seventy two hour period (Figure 5) Thereafter the number of bacterial cells slowly decreased and in most animals the blood was sterile at the end of the five day observation period Animals receiving cortisone or hydrocortisone evidenced a positive blood culture somewhat earlier than the control rabbits and in these animals the bacteraemia increased so rapidly that within twenty four to forty eight hours there was overwhelming sepsis Essentially the same results were obtained with ACTH treated animals Occasionally infected rabbits treated with ACTH died as early as ten hours following the bacterial injection and in such cases large numbers of pneumococci were found in the blood and tissues

Temperature response It has been reported that cortisone has an antipyretic effect in rabbits given pneumococcal vaccine or a pyrogen isolated from a pseudomonas culture (Recant Ott and Fischel 1950) It will also be recalled that in man one of the striking effects of cortisone in infectious disease is the marked fall in temperature within twenty four hours following the initiation of ACTH or cortisone therapy In the present studies it was found that the febrile response of the infected rabbits was essentially the same in the control and cortisone treated animals This is of interest in view of the considerable difference in the severity of the infection in the two groups of animals (Figure 6) Both groups of animals remained essentially afebrile for the first ten hours following the bacterial injection and thereafter the temperature rose rapidly and was maintained at about 105° F over the next four to five day period The temperature was not measured in the ACTH treated animals

Erythrocyte sedimentation rate In the control animals the erythrocyte sedimentation rate gradually increased reaching a maximum of about 40 mm per hour six days following the infection and thereafter gradually returned to normal within the next

few days The sedimentation rate in the treated animals increased rapidly and reached a peak at the time of death This value in the treated animals averaged 125 mm per hour (Figure 7) The erythrocyte sedimentation rate appeared to parallel the severity of the infection

Blood picture studies The blood picture in normal control rabbits treated with saline was quite uniform except for the eosinophils which occasionally disappeared from the peripheral blood for reasons not known at present In the non infected animal, cortisone produced a lymphopenia, eosinopenia and a granulocytosis The lympho penia and granulocytosis occurred simultaneously and hence the total white blood

TABLE I

Haematology Studies in Non Infected Cortisone Treated Rabbits

Time in Hours	Haemoglobin	Total Cells Per mm³			
		WBC	L	PMN	E
0*	14 0	11,000	6 534	3,878	332
12	14 0	11 200	6 734	3 876	334
60	11 5	9,000	3 780	4,770	0
72	11 5	9 400	2,914	6,016	0
84	11 5	10,300	3 296	6,695	0
96	11 0	10,500	3,570	6 930	0
108	12 0	9 800	3 069	6 731	0
116	11 5	10,000	2 828	6,767	0
132	11 0	10,700	3 200	6 890	0
140	11 0	10,870	3,587	6 956	0
156	11 0	9,250	2 812	5 735	0
170	11 0	10 200	2 346	7,548	0
228	11 0	11,080	3 765	6 758	0

* Cortisone started—10 mg per rabbit per day
L = Lymphocytes PMN = Polymorphonuclear neutrophils E = Eosinophils

cell count was essentially unchanged (Table I) Cortisone also produced a mild normocytic hypochromic anaemia in these rabbits

In the infected saline treated control rabbits a leucocytosis developed which had its onset within forty eight hours after the pneumococcal injection and reached a peak of 23,750 white blood cells per cu mm in ninety six hours (Table II) The differential counts revealed a moderate transient depression in lymphocytes and a rise in granulocytes during the foregoing leucocytosis Moreover, the eosinophils were found to disappear from the peripheral blood within seventy two hours after the bacterial injection A possible explanation for the lymphopenia and eosinopenia might be presented on the basis of increased adrenal cortical hormone secretion brought about by the stress of infection

When the animals receiving cortisone were infected, a transient, mild leucocytosis also appeared shortly after infection, but within forty eight hours after the bacterial injection the count returned to normal and remained normal until shortly before death when all blood elements fell to a subnormal level as frequently reported to occur in cases of overwhelming sepsis (Table III) The white blood cell differential studies in these animals revealed essentially the same picture of a lymphopenia and granulocytosis as reported for the non infected cortisone treated animals

TABLE II

Haematology Studies in Saline Treated Infected Rabbits

Time in Hours	Haemoglobin	Total Cells Per mm ³			
		WBC	L	PMN	E
0	14	11 560	7,860	3 136	231
12	14	11 800	7 920	3 200	220
24	14	12 100	7,623	3 751	121
36	14	12 200	6 890	4 680	390
48	14	11 100	6 438	3 885	111
Hours Post Infection			Infected		
0	13 5	12 400	7 316	4,464	124
6	14 0	9 900	4 554	4 752	99
18	13 5	11 000	5 500	4 510	110
24	13 5	11 200	5 488	4 256	112
48	13 0	13 300	6 118	5 712	266
72	14 0	15 850	9 510	4 818	0
96	14 0	23 750	9 737	11 946	0
120	12 0	15 500	6 975	7 750	0

L = Lymphocytes PMN = Polymorphonuclear neutrophils E = Eosinophils

EFFECT OF CORTISONE ON OTHER DEFENCE MECHANISMS

Virulence and pathogenicity of pneumococci Frequent examination of the pneumococcal cells isolated directly from cortisone treated animals dying as a result of overwhelming generalized infection showed that the pathogenicity and virulence for rabbits or mice were not altered by cortisone Pneumococci grown in blood containing cortisone or cultured in blood obtained from rabbits undergoing intensive cortisone treatment were identical with the parent cells insofar as morphology staining properties or virulence were concerned Therefore the altered response of ACTH and cortisone treated rabbits to pneumococci does not appear to be related to changes in the pneumococci

Phagocytosis Phagocytosis by the macrophages or fixed histiocytes of the reticulo endothelial system represents a major feature of the host's defence against infection When a moderately heavy inoculum of Type I pneumococci was injected intravenously into normal rabbits the bacterial cells were rapidly cleared from the blood and the animals survived the infection When cortisone treated animals were given a similar bacterial injection, some of the pneumococci were removed from the blood but apparently many bacteria escaped destruction and began to multiply and

TABLE III

Haematology Studies in Cortisone Treated Infected Rabbits

Time in Hours	Haemoglobin	Total Cells Per mm³			
		WBC	L	PMN	E
0*	13 0	13,280	7,825	4,640	265
12	12 6	13 300	7,845	4,600	225
24	11 0	11 700	5 150	4,740	0
36	11 0	13,000	4,290	8 060	0
48	10 5	12 960	3 615	9,222	0
Hours Post Infection			Infected		
0	10 0	12,200	3 290	8,630	0
6	11 0	10 720	3,220	6 765	0
18	10 5	12,430	3 740	8 460	0
24	10 5	15 450	4 160	10 855	0
48	9 5	9 830	2,260	7,274	0
72	10 5	9 350	2,150	7,106	0
96	10 0	9 600	1,008	8,400	0
120	6 0	4 600	322	3,910	0

Cortisone started—10 mg per rabbit per day
L = Lymphocytes PMN = Polymorphonuclear neutrophils E = Eosinophils

reappear in large numbers in the bloodstream (Figure 8) This eventually led to a generalized infection and death However, if the animals were passively immunized by the intravenous injection of small quantities of Type I pneumococcal antiserum, the cortisone treated animals were capable of rapidly removing and destroying the pneumococci

Development of protective antibodies Studies concerning the effect of cortisone on antibody formation seemed of particular interest in view of the possible effects of cortisone on lymphocytes and the suggested relation of the latter to the formation of antibodies It has been reported that under certain conditions cortisone will depress antibody formation (Bjorneboe, Fischel and Stoerk 1951) The results of the present

study confirm previous reports and show that there was a definite delay in the ap pearance of protective antibodies in the sera of the cortisone treated infected rabbits (Figure 9)

Effects of smaller doses of cortisone Having established that moderately large doses of cortisone will lower resistance to this infection it seemed important to determine the effects of smaller doses Doses ranging from 0 005 mg to 10 mg per rabbit per day were given subcutaneously once daily as in previous experiments and treatment was initiated five days before the pneumococcal injection Observations were made regarding the skin lesion size onset and degree of bacteraemia and mortality With doses smaller than 2 5 mg per rabbit per day, the results are variable insofar as the skin lesions are concerned (Figure 10) However, the blood culture studies reveal that doses smaller than the 0 5 mg level may even enhance the resistance of rabbits to this infection since the bacteraemia with the smaller dose levels was of shorter dura tion and smaller magnitude than that in the control animals (Figure 11) The sig nificance of these findings is presented in the discussion

Chemotherapy in cortisone treated animals It has been clearly shown that large doses of cortisone lower the resistance of rabbits to a pneumococcal infection and that this deleterious effect involves both cellular and humoral mechanisms Chemothera peutic agents such as penicillin are known to exert their effect directly on the invad ing micro organisms and do not require the participation of the host mechanism except for the important subsequent step of eliminating bacteria which have been inhibited but not destroyed In view of the practical significance of this problem it seemed important therefore to determine if the cortisone treated animals could combat infection successfully when the impaired defence mechanism was supple mented with penicillin When doses of 3 000 units of penicillin G per day were injected subcutaneously into cortisone treated rabbits infected with pneumococci in the usual manner all the rabbits survived and there was no evidence of infection as judged by the skin lesion and blood culture studies (Figure 12) Therefore penicillin will eliminate infection in cortisone treated animals despite the impaired host response Studies to be reported elsewhere show however, that greater amounts of penicillin are required to control infection in cortisone treated animals

DISCUSSION

A review of the literature reveals that ACTH and cortisone exert a deleterious action in certain experimental infections On the other hand thousands of patients suffering from rheumatoid arthritis have been treated with cortisone without evidence of serious complications from intercurrent infection The reasons for the difference in animal and human studies are not known at present although a number of possible explanations are worthy of consideration For the most part the animal studies deal with induced infections and usually large or massive doses of the hormones are administered There are many experimental infections which are not altered by cortisone but frequently negative findings of this type are not published Preliminary animal studies suggest that the effects of cortisone in infectious diseases will vary with the animal species the invading micro organism the pathology of the infectious lesion and the amount of cortisone administered (Robinson unpublished data)

Phagocytosis Phagocytosis by the macrophages or fixed histiocytes of the reticulo endothelial system represents a major feature of the host s defence *against infection* When a moderately heavy inoculum of Type I pneumococci was injected intraven ously into normal rabbits the bacterial cells were rapidly cleared from the blood and the animals survived the infection When cortisone treated animals were given a similar bacterial injection, some of the pneumococci were removed from the blood but apparently many bacteria escaped destruction and began to multiply and

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36	11 0	13 000	4,290	8,060	0
48	10 5	12,960	3,615	9 222	0
Hours Post Infection			Infected		
0	10 0	12 200	3,290	8 630	0
6	11 0	10,720	3,220	6 765	0
18	10 5	12 430	3,740	8,460	0
24	10 5	15 450	4 160	10,855	0
48	9 5	9,830	2,260	7,274	0
72	10 5	9,350	2,150	7,106	0
96	10 0	9 600	1,008	8,400	0
120	6 0	4,600	322	3 910	0

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SUMMARY

1 A survey of the literature indicates that large doses of cortisone, hydrocortisone and ACTH lower the resistance of animals to certain infectious diseases The present study confirms and extends these findings but also shows that small doses of cortisone may have the reverse effect and actually benefit the defence mechanism

2 The adverse effects of large doses of ACTH and cortisone appear to be due to the inability of animals to mobilize polymorphonuclear leucocytes and macrophages at the site of the bacterial injection There also appears to be a depression in the rate of antibody formation and a decreased activity on the part of the histiocytes of the reticulo endothelial system in removing and destroying the pneumococci These effects may be related to the action of these hormones on protein metabolism

3 Penicillin is efficacious in the cortisone treated animal despite impairment of the defence mechanism

4 Much information remains to be obtained regarding the effect of these hormones in experimental and human infection Until such data are available, definite conclusions cannot be drawn regarding the value of ACTH or cortisone in the therapy of infectious diseases To date numerous patients have received ACTH and cortisone for the treatment of rheumatoid arthritis and the number of reports citing the occurrence of intercurrent infection has been negligible

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Therefore, it is not possible to generalize regarding the effect of cortisone on infections Each disease must be treated as a separate entity insofar as the influence of cortisone is concerned

In the case of tuberculosis the data seem fairly clear, and strongly suggest that cortisone and ACTH are contra indicated in this disease However here again nothing is known about the effects of small doses of the adrenal cortical hormones and more experimental information is required before final conclusions can be drawn Preliminary clinical results indicate that patients with severe pneumonia experience a sense of well being and have an increased appetite following ACTH or cortisone treatment (Kass Ingbar and Finland 1950) If such beneficial effects can be brought about without lowering the resistance of the host, these hormones may find utility for the treatment of infectious disease in combination with chemotherapy

The results of the present study show that cortisone hydrocortisone and ACTH definitely lower the resistance of rabbits to a Type I pneumoccocal infection when the hormones are given in doses which produce intense physiological effects The deleterious effects appear to be the result of an alteration in a number of important defence mechanisms of the host These include a decrease in the ability to form antibody, inability to mobilize polymorphonuclear cells and macrophages at a normal rate, and a decreased activity of the fixed histiocytes of the reticulo endothelial system All three factors are of prime importance to the host in combating infection

Studies with small doses of cortisone ranging from 0 1 to 1 0 mg per rabbit per day, indicate that under these conditions this hormone may actually enhance the defence of animals infected with pneumococci These findings are in line with the opinion that some benefit is derived as a result of the adrenal cortical hypertrophy which follows bacterial infection Lurie (1951) has shown that the degree of hypertrophy of the adrenal cortex of natively resistant rabbits is much greater than that of susceptible rabbits similarly infected These findings suggest that there may be an optimal amount of adrenal cortical activity and that at this level, these hormones may aid rather than hinder the defence mechanism

Finally, the present paper shows that penicillin is capable of combating infection in cortisone treated animals even though some actions of the defence mechanism are impaired

In considering the mechanism of action of cortisone and ACTH in infectious disease, a number of findings observed in our animals closely resemble those reported to occur in protein deficient rabbits (Wissler, 1947) Rabbits on a protein deficient diet have been reported to develop diffuse oedematous skin lesions and a depression in circulating antibodies Furthermore, blood culture studies and mortality findings in the protein deficient animals appear to be similar to those observed in our cortisone studies Silber and Porter (personal communication) and Clark (personal communication) have found that the nitrogen loss resulting from cortisone administration is mainly at the expense of peripheral skeletal muscle and skin The nitrogen content of certain internal organs such as the liver, is actually increased These observations suggest that further study concerning the effect of cortisone on protein metabolism may be extremely important in elucidating the mechanism of action of these hormones in infection

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ultimately involved the elbow joint There was no infection at the injection sites and the only antecedent infection was a mild blepharitis with one or two small pustules

I should like to have the views of the meeting as to the source of these metastatic abscesses

H J Robinson The incidence of eye infections after ACTH is extremely low although they do occur I should not have thought that the mild blepharitis in this case could have been the cause of the trouble

The danger to which I referred in connection with eye inflammations was that of the indiscriminate local use of cortisone and ACTH without proper diagnosis

M Reiss When introducing ACTH and cortisone into therapeutic practice, far too little attention has been paid to the dosage It was fortunate that when insulin was introduced its ability to decrease the blood sugar was used as a basis for the therapeutic dose Had insulin been used in the way ACTH is being used at present in many disease entities the use of insulin would have been discredited It is very unfortunate that a basic criterion for their action is still not available in assessing a correct therapeutic dose when cortisone or ACTH is administered

In cases of rheumatism ACTH and cortisone are given in pharmacologic doses which are considerably higher than the amount circulating normally in the body fluids or produced at the time given by the glands in question It would be very interesting to see whether much smaller doses of cortisone or ACTH within the physiological range of these substances as they circulate in the body fluids, do not produce the opposite effect

H J Robinson If time permits I should like to make a few remarks regarding cortisone and intercurrent infection and to present some work which is now in progress concerning the mechanism by which cortisone and ACTH alter host resistance First, with regard to the effects of cortisone and ACTH on intercurrent infection we noted that about 80 per cent of our mice treated with large doses of these drugs developed an infection which was mainly due to *Corynebacterium pseudotuberculosis murium* We discovered that the reason for this was that following the customary laboratory procedures in working with rats we did not use strict aseptic technique and hence we were spreading the infection from a relatively small number of animals which developed an intercurrent infection to all of the animals in a given group When we substituted an aseptic technique or when cortisone was administered orally the incidence of intercurrent infection was markedly reduced

Both the protein deficient and cortisone treated rabbits develop a spreading cellulitis with early and overwhelming sepsis There is evidence that cortisone causes a nitrogen deficiency which is mainly at the expense of peripheral tissues such as skin and skeletal muscle Actually the protein content of certain internal organ such as the liver is increased despite the overall negative nitrogen deficit The question arises as to whether the peripheral protein deficit may account for the suppression of inflammation which in the case of the cellulitis in rabbits is detrimental to the host whereas in the case of rheumatoid arthritis the suppressed inflammation relieves the joint pain associated with this disease

I might also add that our preliminary studies regarding the effect of somatotrophic hormone on intercurrent infection induced by cortisone treatment have not confirmed

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Discussion

Chairman G R Cameron

G R Cameron We have just heard of the fulminating infections that can occur after cortisone and ACTH Fortunately for us, neither the dosage levels nor the infections are comparable with those we meet with clinically, but it should serve as a warning that each patient and each infection should be considered on its merits and that no generalization is possible

J M Yoffey Dr Robinson in your cases did you still get the normal blood changes that one gets with cortisone in spite of the infection?

H J Robinson Yes, cortisone produces essentially the same effects in infected animals as it does in non infected animals

R R H Lovell Inflammation is usually regarded as a defensive mechanism Dr Robinson has shown how this tissue response may be suppressed and the consequence of such suppression Though septicaemia sometimes develops in patients on cortisone or ACTH it is not such a common event as these animal experiments might suggest and the question of hormone dosage may be relevant here In Professor Pickering s unit we have been studying the effects of cortisone and ACTH on experimentally induced inflammations in the human skin Response to tuberculin and to manganese butyrate which is a tissue irritant are both reduced by cortisone and ACTH the tuberculin response being most conspicuously affected The dose of cortisone necessary to produce these changes is about 200 mg daily and of ACTH about 150 mg daily These are higher than are generally used therapeutically

P Ellman I had a case about eighteen months ago which is relevant to this problem She was a woman aged fifty two with polyarthritis bilateral pleural effusion, fever, eosinophilia leucocytosis and a raised erythrocyte sedimentation rate Mr Broster tried an adrenal implant but it was without effect We then gave two small doses of ACTH intramuscularly and she developed multiple metastatic abscesses one of which

were at the beginning of the therapy The disease process may progress without symptoms or inflammatory response under a blanket of hormone There is certainly some X ray evidence that the bone lesions continue to progress whilst the patient is symptomless under cortisone therapy The similarity between the protein deficient and cortisone treated animals in their inflammatory response also would suggest that we are merely suppressing the host s reaction to an injurious agent

H J Robinson The suggestion of mine that cortisone works by causing a tissue protein deficiency was only a theory and has not been proved

The immediate effect of cortisone on rheumatoid arthritis patients is without doubt beneficial and welcomed by the patient

G R Cameron We are certainly debating the most debatable of topics in this discussion We are still at the fact gathering stage and it is impossible as yet to make dogmatic statements

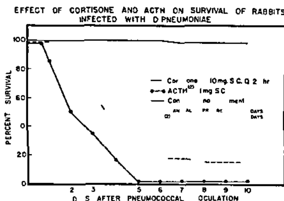


Figure 1

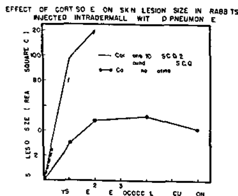


Figure 2

the report of Selye This, however, may be due to the presence of different flora in our animals and also to a difference in the somatotrophic hormone

G R Cameron With regard to this danger of secondary infection with cortisone treatment One of my staff has been doing some experiments on the healing of tendons in rats His operative technique was such that he never got infection of the wound site When he gave cortisone to these animals in large dosage, many showed infection at the operation site We have also had an epidemic of typhimurium infection in rats treated with cortisone

The bacteriaemia one sometimes gets with cortisone might suggest that there may be some change in the reticulo endothelial system or, perhaps, in the lymph node Is there any evidence of this?

H J Robinson None that I know of but perhaps Professor Yoffey can help us with this

I have carried out some experiments with an isolated perfused spleen We inoculated micro organisms into the perfusing fluid and measured the rate of removal of the micro organisms from the fluid before and after the addition of cortisone Cortisone produced its effect within an hour which is probably too short for a pathological change so it may be merely a functional one

J M Yoffey The problem is probably, in part, that of the freedom with which bacteria can move in the connective tissues If they can move freely, they will enter lymphatics and after passing through one or more lymph glands reach the blood stream Drinker and his colleagues, some years ago, were faced with an analogous problem in connection with the spread of type IV pneumococci in the rabbit They found that after dropping suspension of these pneumococci into a rabbit s nose they rapidly passed through the mucosa, entered the cervical lymphatics and passed through one or more glands to enter the blood From the blood they passed through the walls of capillaries in various parts of the body to reach the connective tissues and thence via the lymphatic vessels and lymph glands found their way back to the blood The connective tissue, lymph vessels and glands thus formed an extravascular reservoir from which the blood was being constantly re infected even though for a time the blood could be emptied of pneumococci by giving anti serum In these particular experiments the easy spread of the type IV pneumococci was a property depending on their virulence In the case of cortisone the spread of bacteria is presumably facilitated by the action of cortisone on the connective tissues and not by augmentation of bacterial virulence

J T R Duthie This is the first session of the Symposium at which pharmacological studies of the effect of ACTH and cortisone on inflammation have been discussed We must beware of this possibly artificial distinction between the Collagen diseases and inflammation the cause of which we know It seems to me that when we do not know the cause of an inflammatory process, we say this process is bad and must be suppressed, but when we do know the cause we say that this is a benign reaction and should be encouraged It may be that the inflammation in the former group may be beneficial to the patient and we may be depriving him of his defence reaction I have been much concerned with the condition of rheumatoid arthritis patients after withdrawal of the hormone therapy Many of them seem to be in a worse state than they

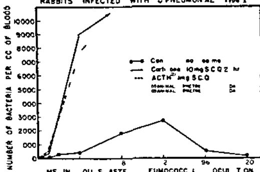


Figure 5

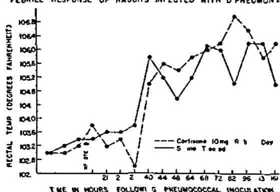


Figure 6 Cortisone treatment begun five days prior to infection and continued once daily until death of the rabbit Rabbits infected intradermally with 0 1 *cc of a six hour culture of* D pneumoniae *Type I*

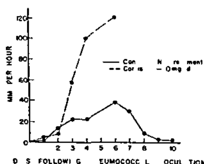


Figure 7 Cortisone treatment begun five days prior to infection and continued once daily until death of the rabbit Rabbits infected intradermally with 0 1 *cc of a six hour culture of* D pneumoniae *Type I*

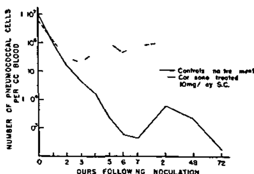


Figure 8

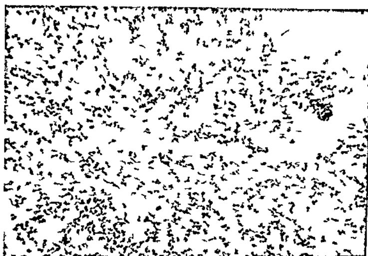


Figure 3 Photomicrograph of inflammatory reaction in control rabbit twelve hours after bacterial injection Magnification 440 ×



Figure 4 Photomicrograph of inflammatory reaction in cortisone treated rabbit twelve hours after bacterial injection Magnification 440 ×

*Adrenal Steroids and Personality Disorders**

by

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SOME CONSIDERATION OF BRAIN FUNCTION IN RELATION TO THE ACTION OF ADRENAL STEROIDS

A CONSIDERATION of possible effects of adrenal steroids on mental disorders might well start with an inquiry into what action if any these substances exert on brain physiology Without attempting a systematic review of this subject I would like to describe some recent unpublished experiments from our laboratory relevant to this matter It had previously been demonstrated independently by Gordan *et al* [1] and by Hayano and Dorfman[2] that certain steroids in relatively large amounts inhibit the oxygen consumption and glucose utilization of rat brain homogenates through action on specific flavoprotein entities of the electron transfer system

However Bergen and Hunt in our laboratory in extensive studies of rat brain homogenates and slices from normal and from adrenalectomized rats found no group differences in *in vitro* oxygen consumption The inhibitory effects in so far as they are produced by adrenal steroids were not seen in the normal physiological range in these experiments It is well known that the frequency of the electroencephalogram is reduced in Addison s disease and restored by cortisone administration[3] and Bergen[4] has demonstrated that adrenalectomy slows the EEG of the unanaesthetized rat and that this slowed frequency is restored three hours after injection of adrenal cortical extract Since slowing of the EEG may result from decreased oxygen and glucose utilization of the brain[5] [6] it occurred to us that the 11 oxysteroids may have effects *in vivo* not detectable by *in vitro* experiments and that a key to this problem might reside in the action of the adrenal steroids on the cerebral circulation Levine *et al* [7] have demonstrated that adrenal cortical extract re establishes a more normal blood flow in adrenalectomized animals and thus facilitates the work output of muscle *in vivo*

It seemed possible therefore that the slowed EEG of adrenalectomized animals kept in good condition on salt might result from slowed cerebral circulation and accompanying oxygen deprivation and data have been obtained by John Bergen and Charlotte Hunt to test this hypothesis Our *in vivo* studies indicate a reduction in blood flow through the brain of 61 per cent in adrenalectomized rats and show that

* The studies of adrenal steroid metabolism and physiology discussed in this paper have been aided by a co-operative project with the National Institute of Mental Health U S Public Health Service Federal Security Agency by a contract with the Office of Naval Research and by grants from the Williams Waterman Fund of the Research Corporation The Scottish Rite Committee for Research on Dementia Praecox The George I Alden Trust The Theodore Edson Parker Foundation Grant No 2563 of the physiology section of the U S Public Health Service and by the co-operation of the Research Service of the Worcester State Hospital

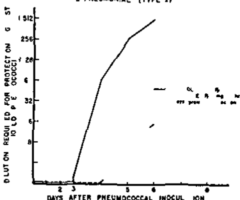


Figure 9 Circulating antibodies determined by mouse protection test (12)

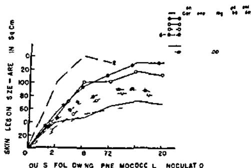


Figure 10 Cortisone treatment begun five days prior to infection and continued once daily until death of the rabbit Rabbits infected intradermally with 0 1 cc of a six hour culture of D pneumoniae, *Type I*

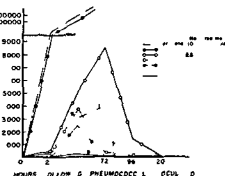


Figure 11 Cortisone treatment begun five days prior to infection and continued once daily until death of the rabbit Rabbits infected intradermally with 0 1 cc of a six hour culture of D pneumoniae, *Type I*

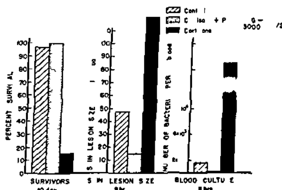


Figure 12 Cortisone treatment begun five days prior to infection and continued once daily until death of the rabbit Penicillin G was administered from time of infection Rabbits infected intradermally with 0 1 cc of a six hour culture of D pneumoniae *Type I*

apparently an important intermediate in the synthesis of the adrenal cortical hormones from cholesterol ACTH is necessary for the conversion of cholesterol to Δ5 pregnenolone which then at a rapid rate and without further action of ACTH, is converted to progesterone by modification of ring A

Progesterone itself is rapidly converted along several pathways to a variety of corticoids including among others DCA compounds A, B E and F In the case of the restorative action of Δ5 pregnenolone on brain blood flow brain oxygen consumption and the EEG of adrenalectomized rats the effect is probably a direct one since we know of no tissue other than the adrenal capable of converting this substance to oxysteroids of the adrenal type

Slocombe in our laboratory has recently made cathode ray oscillograph records of the primary electrical response of the rat somatic sensory cortex following electrical

TABLE II

Brain blood flow with standard errors and median brain wave frequencies in normal and adrenalectomized rats and in adrenalectomized rats given replacement therapy

	No of rats	Blood flow ml/min		Diff from controls ml/min	Sig of Diff P	No of rats	EEG median freq ~/sec	Diff from controls ~/sec	Sig of diff P
Normal	8	1 65	±0 12	—	—	130	44 0	—	—
Adrx	19	0 65	±0 71	1 00	<0 005	116	37 8	6 2	<0 01
Adrx plus ACE	17	1 69	±0 14	0 04	not sig	1	42 2	1 8	not sig
Adrx plus pregnenolone	13	1 34	±0 11	0 31	not sig	21	42 7	1 3	not sig
Adrx plus DCA	16	1 05	±0 15	0 60	<0 01	23	36 5	7 5	<0 01

stimulation of the foot Adrenalectomized rats kept in good condition on salt show in comparison to unoperated controls slowing of conduction time of this response by 19 per cent Table III illustrates these data The difference of 3 6 ms in conduction time is primarily due to central delay since a 19 per cent reduction in velocity of conduction in the peripheral nerve could account for only approximately 1/4 ms of this difference The slowed conduction is restored after three days of daily injection of the adrenalectomized rats with 0 25 ml of lipoadrenal extract (equivalent to 0 25 mg compound F) Slocombe also finds that the excitability of the sciatic nerve *in situ* is decreased about 150 per cent by adrenalectomy as measured by increase of Hill s excitation time constant The excitability is partially restored three hours after injecting adrenal cortical extract

In summary these studies with rats indicate that timing of events in the nervous system as measured by synaptic delays of the somatic sensory response and of the circuits mediating the EEG are effected by the oxycorticoids (and by Δ5 pregnenolone)

the brain receives only 54 per cent. of the normal amount of oxygen in these animals. This corresponds to a reduction of 46 per cent. in brain oxygen consumption and the differences are significant ($P<0.005$). According to Greene[8] the posterior facial veins of the rat drain the brain sinuses. The rate of blood flow from the left anterior facial vein was measured after ligating venous return from the head excepting the vertebrals. Arterial venous differences in blood oxygen content were made by the Roughton and Scholander method. While more oxygen is removed by the brain from the slowly flowing blood in the adrenalectomized rats the total reduction in blood flow is sufficiently great *substantially to reduce the tissues' oxygen supply.* (See Table I.) The administration of 0.50 ml. of Upjohn's lipoadrenal extract (equivalent to 0.50 mg. of Compound F) to adrenalectomized rats restores completely the blood flow and oxygen consumption to normal in three hours. The blood flow is restored by half of this amount of the extract but this smaller dose was not tested on the EEG. We also found that 250 gamma of Δ5 pregnenolone restores brain blood flow and oxygen consumption, but DCA even in twice this dose has only a small restorative

TABLE I

Brain blood flow, arterial and venous oxygen values and standard errors in normal and adrenalectomized rats kept in good condition on salt

	No. of rats	Blood flow ml/min	No. of rats	Arterial oxygen vol. per cent	Venous oxygen vol. per cent	A-V diff. vol. per cent	Oxygen consumed per min	Per cent decr. with adrx
Normal rats	8	1.65 ± 0.1	10	14.9 ± 0.64	8.13 ± 0.48	6.8	0.113	—
Adrx. rats	19	0.65 ± 0.071	9	14.8 ± 0.80	5.4 ± 0.89	9.4	0.0610	54

action. Cortisone (250 gamma) also restores brain blood flow and oxygen consumption to normal in three hours.

Table II and Figure 1 summarize some of these findings and indicate that the EEG is slowed in adrenalectomized rats as a result of anoxia brought about by reduced brain circulation. This is seen from the parallelism between the action of substances that restore blood flow and correspondingly restore the EEG. The restorative action of cortisone on blood flow and of Δ5 pregnenolone and adrenal cortical extract (ACE) on oxygen consumption are not shown in the figure or table since these results were obtained just before my departure for this conference.

The restorative action of Δ5 pregnenolone on the EEG is of special interest since we[9, 10, 11] have shown that this substance when taken by mouth has anti-fatigue action *in man and significantly facilitates psychomotor performance.* This was demonstrated in studies of several hundred factory workers and aviators engaged in exacting and measurable psychomotor tasks when taking 50 to 100 mg. per day of Δ5 pregnenolone in comparison to their performance on placebos. Δ5 pregnenolone is not an alpha ketol. It has a methyl group at C 21, an hydroxyl at C 3 and a double bond between carbons 5 and 6. It lacks oxygen at C 11 and C 17. Perfusion studies of the beef adrenal carried out in our laboratory[12] have shown that Δ5 pregnenolone is

have not been specifically identified but probably include androstanediol pregnane diol and etiocholanediol

Figure 3 illustrates the lesser output of urinary adrenal corticoids in schizo phrenics measured both as neutral reducing lipids and as formaldehydogenic sub stance Work from several laboratories has shown that the total corticoid output of arthritic patients is not different from that of normal controls, and in this figure the arthritic and schizophrenic patients belong to comparable age groups Follow ing administration of 100 mg of cortisone daily for one week we see that the controls show an increased output of corticoids in terms of both types of measures but no significant changes are seen in the corticoid output of the schizophrenic patients We think that this indicates the utilization of the added cortisone by the patients tissues leaving no excess of alpha ketols for excretion The low output of corticoids by the patients suggests this interpretation

Chromatographic analyses (unpublished) of urinary corticosteroid output show that schizophrenics produce in comparison to controls a higher percentage of polar corticoids about the same percentage of corticoids of the corticosterone and cortisone type and a relatively smaller percentage of the less oxygenated corticoids We refer here to percentages of the total corticoids compared to percentages of normal sub jects It should be recalled that the total output of corticoids by the patients is less than that of normals

ADRENAL STRESS RESPONSES OF SCHIZOPHRENIC AND NORMAL MEN

When schizophrenics are experimentally stressed and relative per cent changes from pre test levels are measured in 17 ketosteroids urinary sodium potassium uric acid and urinary phosphate as indices of adrenal response we found that approximately two thirds of the patients are relatively unresponsive compared to criteria established by normal persons undergoing the same stresses[14]

Our standard stress tests have involved the ingestion of sugar by the Exton Rose sugar tolerance test procedure the operation for one hour of a pursuitmeter and a psychological frustration test the Rotter Rodnick target ball test[14] Urine and blood samples were collected before the test and immediately after (at the one hour period) and again two hours later (three hours from the start of the test) Responsivity of our adrenal indices to injected lipoadrenal extract have shown no significant differences between schizophrenic patients and normal controls indicating that tissue meta bolism in response to the action of the steroids is not the source of the difference in the stress responses A comparable degree of unresponsivity is also seen following standard test injections of ACTH thus indicating that the defect lies at the level of the adrenal cortex itself These findings of responsivity to ACTH of schizophrenic patients are in general agreement with those of Hemphill and Reiss[18] and have been confirmed by Faurbye Vestergaard *et al* [19] Hospitalized psychoneurotic patients have been shown by us and by others in studies of adrenal responsivity to stress and to injected ACTH to display normal responses[14] o that hospitalization *per se* is not the factor at issue These findings are consistent with the view that the schizophrenic patients adrenals produce abnormal types of steroid substances and that their adrenal responsivity both to endogenous and to injected ACTH is abnormal The abnormal

ADRENAL CORTEX FUNCTION IN NORMAL AND PSYCHOTIC MEN

In recent years a number of investigators at the Worcester Foundation have been engaged in a series of studies of the response of the normal human adrenal cortex to workaday stresses We have demonstrated that such stresses as flying aeroplanes, operating machines, the taking of examinations, exposure to heat and cold, and the ingestion of sugar result in an enhanced output of adrenal cortical hormones as measured by the excretion of urinary corticoids 17 ketosteroids, potassium, sodium and uric acid [13 14 15 16] Accompanying these urinary changes following the stress are characteristic declines in circulating lymphocytes and eosinophiles resulting from the heightened activity of 11 oxycorticoids This very general response to quite non specific and often mild stresses has indicated to us fluctuations in the demand of tissues for steroid hormones as a part of one s adjustment to the social and physical environment

Schizophrenic patients are persons who have notably failed to meet life's day to day stresses and have developed bizarre behaviour patterns which usually require

TABLE III

Conduction Time Foot to Somatic Sensory Cortex

	No of rats	Cond Time (ms) and S E	Per cent increase above normal	t	P
Normal	9	19 2 ±0 85	—	—	—
Adrx	15	22 8 ±0 98	19	2 74	0 02

hospitalization Nine years ago in collaboration with investigators at the Worcester State Hospital, we began a series of experiments which are still continuing to examine the adrenal cortical physiology of schizophrenic patients with the thought of testing their responses to some of the same stresses we had used with our normal subjects Approximately two hundred male patients and three hundred normal male controls have been examined in a variety of test situations

Compared to normal controls of the same age levels the patients excrete, under fasting conditions, on the average approximately the same amount of total 17 ketosteroids, potassium, uric acid and creatinine but excrete only about two thirds of the normal amount of corticoid (C-21) steroids and an excessive amount of sodium and urinary water [14] The patients excrete only about one half of the normal amount of inorganic phosphate,[16] and only about two thirds of non ketonic steroid substances compared to normal controls [17]

Figure 2 shows the marked reduction of urinary non ketonic steroids produced by nineteen schizophrenic patients in comparison to thirteen normal controls of the same age range These non ketonic steroids measured by the $SbCl_3$ colour reaction,

In view of these considerations it occurred independently to Dr Charles Huggins of the University of Chicago and to us that total adrenalectomy of schizophrenic patients and their maintenance on cortisone and salt might prove to be a therapeutically beneficial procedure Huggins and Bergenstal[25] had successfully adrenalectomized patients suffering from prostatic and breast cancer and in thirty cases to date they have encountered no adrenal insufficiency when the patients take 25–50 mg of cortisone per day by mouth and 3 to 4 grams of salt tablets As Dr Huggins has aptly phrased it he proposed to remove possible sources of abnormal adrenal hormones from the schizophrenics and substitute a normal adrenal balance

Six chronic schizophrenic patients were accordingly selected by Dr Nathaniel Apter and Dr Maurice Lipton of the Psychiatry Department at the University of Chicago family permissions were obtained and total adrenalectomies were performed in Chicago in September 1951 by Huggins and Bergenstal These patients since maintained on oral cortisone and salt are all in excellent physical health and metabolic balance at the present time The patients have been under careful psychiatric and psychological observation by Doctors Apter Lipton and Ward Halstead from several weeks before the operation to the present time

Dr Eli Romanoff of the Worcester Foundation was present at the operations and mounted the adrenals in a perfusion apparatus immediately after their removal perfusing them for eight to ten hours with human blood Test injections of ACTH were also administered to the glands and the ability of the adrenals to convert certain steroids to 11-oxysteroids was also tested The perfusates are being studied and compared in our laboratory by paper chromatography and other procedures with perfusates obtained from other human adrenals taken from non schizophrenic subjects for the relief of cancer and hypertension By these and similar perfusion studies we believe it will ultimately be possible to be quite specific about the nature of steroidogenesis of schizophrenic patients

At this time we are not prepared to discuss the results of the perfusion studies or the psychiatric effects of the procedures These latter will be reported upon at a later date Suffice it to say that the results are sufficiently encouraging in the opinion of all of those concerned to warrant extending this operative procedure to other schizophrenic patients

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responses are not corrected by feeding the patients a protein and vitamin rich diet[20] In passing we wish to emphasize that in the three stress tests described above and in our test injections of ACTH and of adrenal cortical extract we have not found per cent changes in lymphocytes or eosinophiles or corticoids to differentiate between patients and normals Our group differences in this series are reflected only in per cent changes in the urinary variables other than corticoids

Broster and Allen[21] have described cases of hyperadrenalism in patients diagnosed as schizophrenic who some months after the removal of one overactive adrenal, showed complete remission of symptoms Psychosis as we mentioned has frequently been described as a consequence of Cushing s syndrome and the adrenal genital syn drome and as a result of overdosage with ACTH and cortisone in patients hitherto non psychotic[2] We have found that the more normal the responsivity of schizo phrenics' adrenals to ACTH the better their subsequent prognosis when given electro-shock therapy [3] As we have seen, adrenal steroids normally exert action on the cen tral nervous system *in vivo* as measured by aspects of its electrical activity and its metabolism In view of all of these observations it has seemed to us that the over production of abnormal steroids or their metabolites may play an important role in the aetiology of schizophrenia, and this hypothesis is now under investigation

We wish to make it clear that we do not believe that aberrant adrenal physiology is necessarily the cause of schizophrenia Rather we think it may be one of several important factors involved in its aetiology Other types of psychoses may also involve disturbances in adrenal function We have found some evidence for this in a study of ten women suffering from involutional depressions[24] These patients showed improve ments of adrenal responsivity following successful electroshock therapy Hemphill and Reiss[18] have also presented evidence of abnormal adrenal physiology in psycho tics other than schizophrenics If abnormal adrenal stress responsivity exists before the development of symptoms it may constitute a predisposing factor for the disease Psychosis may never develop in such persons if their lives present few problems but in the face of life s batterings they may be unusually vulnerable to this form of per sonality disturbance It is of course possible that the adrenal abnormality occurs concomitantly or secondarily to the development of psychosis Our data at present cannot settle this matter

CONTINUING STUDIES

I would like here to emphasize several matters reviewed above

(1) Schizophrenic patients display abnormal steroidogenesis as reflected by their urinary data

(2) Their adrenal responsivity is subnormal both to stress and to injected ACTH

(3) Some persons not hitherto psychotic develop psychoses following prolonged over-dosage with cortisone and ACTH

(4) Psychosis sometimes accompanies adrenal hyperplasia and is relieved follow ing removal of one overactive adrenal

(5) Patients with adrenals more normally responsive to ACTH have a better prognosis when given electroshock therapy

(6) Animal studies show that adrenal steroids help regulate central nervous processes in terms of timing of nerve messages via synaptic conduction

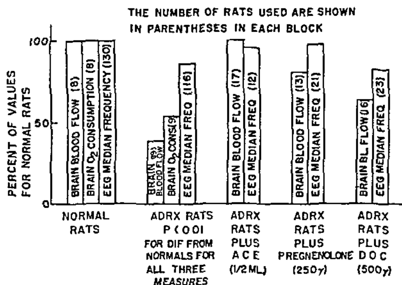


Figure 1

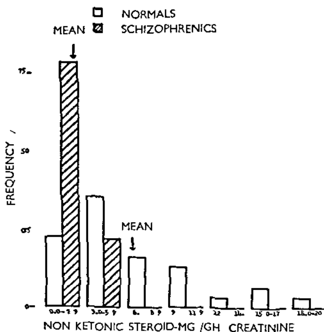


Figure 2

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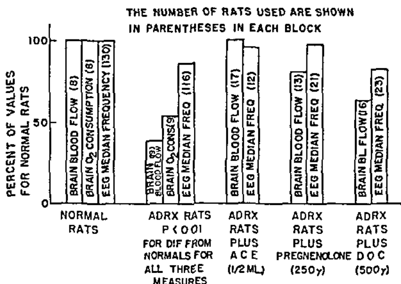


Figure 1

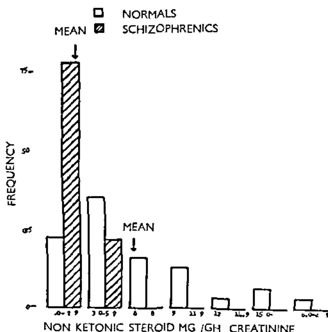


Figure 2

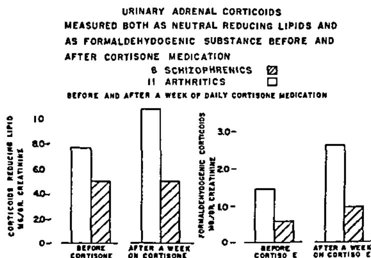

URINARY ADRENAL CORTICOIDS
MEASURED BOTH AS NEUTRAL REDUCING LIPIDS AND
AS FORMALDEHYDOGENIC SUBSTANCE BEFORE AND
AFTER CORTISONE MEDICATION
8 SCHIZOPHRENICS
11 ARTHRITICS
BEFORE AND AFTER A WEEK OF DAILY CORTISONE MEDICATION
CORTICOIDS REDUCING LIPID MG./GR. CREATININE
10
8.0
6.0
4.0
2.0
0
BEFORE CORTISONE
AFTER A WEEK ON CORTISONE
FORMALDEHYDOGENIC CORTICOIDS MG./GR. CREATININE
3.0
2.0
1.0
0
BEFORE CORTISO E
AFTER A WEEK ON CORTISO E

Figure 3

Changes in Suprarenal Cortex Function in Shock and Hormone Treatments

by

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THIS communication on changes in adrenal cortex function in shock and hormone therapies is a presentation of the work of some of my colleagues (including Dr Max Reiss) and myself at the Bristol Mental Hospitals

The expression shock therapy refers to epileptic convulsions induced by electrical stimulation of brain (a usual method of treatment in psychiatry) or to insulin hypo glycaemic treatment (used principally in schizophrenia) It is necessary to point out that the physiological effects of these shock treatments in normal subjects are totally unknown for these treatments have practically never been applied to volunteers, or if applied occasionally have not been repeated

Since there is evidence of endocrine disturbances in many psychiatric disorders or in phases of them changes in endocrine function produced by shock treatments must be relative that is they may be modified by the endocrine state during the illness preceding the shock The effect of the shock treatment on the endocrine system would depend upon the endocrine condition when it was initiated

The adrenal cortex in psychiatric illness has been studied by Hoagland and his co workers, Altschule Cleghorn ourselves and others In our hospital it has been of interest since ACTH was used therapeutically in involutional melancholia about twelve years ago (Hemphill and Reiss 1942 1944 Hemphill, 1946)

It cannot be claimed that the various modifications of the Thorn test used are entirely satisfactory and we do not pretend that any conclusions drawn will be anything like definite but the present state of knowledge suggests that in a high proportion of cases of chronic schizophrenia there is a degree of adrenal cortex unresponsivity the activity of endogenous adrenal cortex secretion seems often to fail in psychotic subjects and phasic alterations are seen in some cases of manic depressive psychosis

We have investigated adrenal cortex responsivity to ingested glucose and to in jected ACTH in about eight hundred cases It seemed undesirable to combine the fall in eosinophil count the increase in 17 ketosteroids and the alterations in uric acid/creatinine ratio in a single index We have studied the responsivity in the form of eight reaction types (Table I) (Reiss Hemphill *et al* 1951)

In a first series of 360 patients it was seen that no particular response type was specifically related to any psychiatric group (Figs 1 and 2) There were differences between acute and chronic schizophrenia and in male anxiety the adrenal response

TABLE I

Types of reaction to injection of glucose, and injection of ACTH Keto/Cr = ratio of urinary keto steroids to creatinine Uric/Cr = ratio of uric acid to creatinine

Reaction No	Eosinophils	Keto/Cr	Uric Cr
0	—	—	—
1	+	—	—
2	—	+	—
3	—	—	+
4	+	+	—
5	+	—	+
6	—	+	+
7	+	+	+

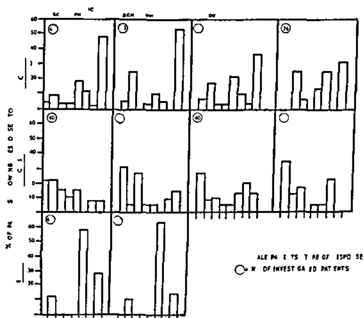


Figure 1

was often worse than in psychotic cases In no single case of chronic male anxiety was there a completely positive response to glucose On the whole disturbances of responsivity to injected ACTH were less frequent and less severe

Some evidence that ACTH of the pituitary lobe might be mobilized by electro convulsive therapy was advanced by us in 1942 (Hemphill Macleod and Reiss) and by Hoagland, Parsons Nikkelsen and others in later years Using response tests we have compared the responsivity of the adrenal cortex to one electrically induced convulsion with the response to 25 mgm ACTH and to ingestion of 150 gm glucose (Fig 1)

Fewer patients show response to one electro shock treatment than to one injection of ACTH Reaction type 5 seldom seen after ACTH and never after glucose, was the most common reaction to ECT A completely negative response did not occur after ECT, and rarely after ACTH Therefore our investigations show that ECT even in schizophrenia, provokes adrenal response

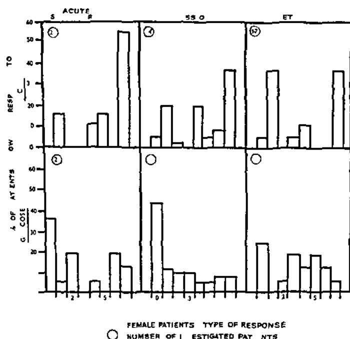


Figure 2

Hoagland *et al* (1950) described in schizophrenia a significant correlation in adrenal response to injected ACTH and to ECT They noted that a subconvulsive electric shock produces an adrenal response and this has raised the question whether convulsion with the circulatory and metabolic changes it implies is essential or whether it is the application of the electric current to the brain This question has yet to be answered

They suggested further that insulin and electro shock represent stresses which act therapeutically by activating endogenous production of ACTH, and consider that prognosis in insulin and electro shock therapy is related to the adrenal cortex responsivity of the patient prior to treatment This is in keeping with our experience

to some extent, but it must be stressed that ECT is practically ineffective in chronic schizophrenia, in contrast to depressive psychosis

Adrenal cortex responsivity, usually constant in healthy individuals, sometimes changes markedly in psychiatric cases as the clinical condition alters Table II shows results in eleven treated and two untreated patients In patient No 1, ECT was ineffective and his adrenal response deteriorated No 7, treated first with ACTH and later with insulin, did not improve, and adrenal response deteriorated No 8, treated with ACTH, did not improve, and response was unchanged No 6, treated with ACTH, improved and response to glucose improved Nos 2, 3 4 and 5 treated with ECT, improved, with corresponding alteration in response tests Nos

TABLE II

Changes in Adrenalresponsivity after Treatment

No	Patient	Sex	Age	Diagnosis	Treatment	Weeks between the two tests	Types of Response to ACTH		Glucose		Clinical change between the two tests
							Before	After	Before	After	
1	M M B	F	66	Paranoid reaction	ECT	7	1	0	0	0	No improvement
2	A F W	M	42	Schizophrenia	ECT	12	1	7	0	0	Improved
3	L I S	F	45	Depression	ECT	4	7	7	0	6	Improved
4	S V M	M	24	Neurosis	ECT	6	7	6	0	6	Improved
5	G E L	F	61	Depression	ECT	5	3	7	2	6	Improved
6	A M K	F	30	Depersonalization	ACTH	6	7	–	0	5	Improved
7	P B	M	26	Chronic schizophrenia	ACTH and insulin	36	7	5	7	0	No improvement
8	W E C	F	52	Depersonalization	ACTH	10	5	5	3	3	No improvement
9	E L D	F	36	Depression	Testosterone implant	8	7	7	0	7	Improved
10	L R	F	62	Depression	Testosterone	10	5	7	1	6	Improved
11	I M B	F	42	Depression	Testosterone	16	1	7	0	3	Improved
12	W T R	M	17	Schizophrenia	No treatment	6	1	5	0	7	Spontaneous improvement
13	P H J	M	42	Depression	No treatment	10	1	7	0	0	Spontaneous improvement

9, 10 and 11, treated successfully with testosterone, showed increased adrenal cortex responsivity

We have carried out determination of ACTH content in blood of patients (using Sayers's method) before and after ECT Within the reliability of the method, it would appear that changes in ACTH blood content three minutes after electro shock are most irregular Table III shows how there may be an increase in ACTH three minutes after ECT, or in some patients an increase after the first shock and a decrease after the second there are patients in whom there is a decrease after the first shock Thus to study adrenal cortex function alone cannot throw much light on the mechanism of shock treatments but the mode of action becomes clearer when the endocrine relationships and total endocrine equilibrium are considered

Response tests themselves may have a therapeutic effect either through the mobilization of ACTH endogenously or by the push given to the endocrine equilibrium by injected ACTH used in the test dose, and this limits their usefulness We have observed one case of mild recurrent manic depressive psychosis in which the phase of depression was immediately altered in the direction of normality or mania by a single test injection of 50 mgm ACTH and we have had experience of a small number of cyclothymic patients in the mildly depressed phase whose attack was terminated in the same way This appears to represent something of the effect of a minimal electro shock treatment, adequate for altering a minor depressive phase but ineffective in a more severe disturbance

TABLE III

ACTH content of blood before and after ECT

Patient	No of ECT	mU ACTH per 100 ml Blood	
		Before ECT	3-5 min after ECT
Mrs E V	1st 3rd	7 120	110 50
Mrs V K	1st 2nd	4 42	14 0
Mrs P	1st 2nd 3rd	0 0 0	0 8 70
Mr H	1st	30	5
Mrs H	1st	9	0

Adrenal function in depression in relation to the intervals between electro shock treatments has not been studied adequately Normally the first four or six treatments are given at intervals of a day or two and relapses are usual if the interval is too long Repeated applications of ECT at intervals of more than a week may lead to reduction of adrenal responsivity In one case of unequivocal periodic depression who had been treated in this way and in whom ECT was without effect the response to injected ACTH and to glucose was exceptionally poor

In spite of the disadvantages determination of the pituitary adrenocortical responsivity has at present an important place in psychiatric research The tests seem to give information about the state and seriousness of the clinical condition and permit some prognostic conclusions and the results can be used as an objective criterion in therapeutic research against which one judges improvement

The results communicated allow some conclusions to be drawn about endocrine physiology The fact that electro shock may produce qualitatively different adreno cortical response from injection of ACTH or ingestion of glucose suggests that different ACTH fractions are mobilized by these different agencies There appears to be a separate fraction mobilizing reducing neutral lipoids and a separate fraction mobilizing 17 ketosteroids

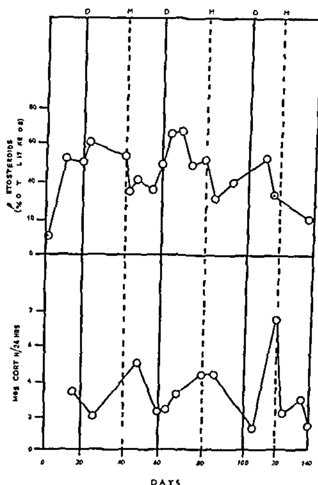


Figure 3

In some patients, changes in reducing neutral lipoid and 17 ketosteroid excretion can take place independently of each other The excretion curves in Fig 3 relate to a manic depressive patient, in whom reducing neutral lipoids and β ketosteroids were inversely related the peaks of the former occurring in mania and the peaks of the latter in depression (Reiss Hemphill *et al* 1949)

The easy assumption that psychological stress influences or impairs adrenal function and produces thereby an alteration in the psychiatric picture is difficult to justify Inactivity may constitute a mental stress in some patients and activity in others

It appears to us that the hallucinations that disturb the acute schizophrenic are a form of stressful thinking while the indifference displayed by the chronic schizophrenic to apparently the same psychological disturbances is in keeping with an impaired adrenocortical responsivity Many schizophrenics seem unable to preserve a stable endocrine balance as indicated by day to day variations in ketosteroid output and variations in adrenal responsivity This is in our experience a bad prognostic sign, and no doubt accounts for some of the contradictory results found by every worker

The point has been reached when it is necessary to define more precisely the psychiatric criteria for illnesses under investigation, to determine more precisely than formerly the psychiatric response to environmental changes and to develop methods in which the subjective element is less prominent than at present

So far all research workers have accepted the fact that we are practically ignorant of the significance of the duration of psychiatric illnesses and we have no means of measuring their severity The terms acute and chronic are used for convenience but so loosely and with so much overlapping that they are almost without meaning except at the extremes

SUMMARY

1 Various forms and degrees of disturbance of adrenal cortex responsivity are found in psychiatric illness

2 Poor responsivity is often associated with a poor prognosis and *vice versa*

3 Electro convulsive and other shock therapies do influence responsivity of adrenal cortex but in a complex and variable way about which we can only speculate

4 ECT produces considerable alterations in the ACTH levels in blood measured by Sayers s method but its effect is highly variable and the level in some cases may even be reduced

5 ACTH is not a therapeutic substitute for ECT but in some cases of mild periodic depression it appears to operate in a similar fashion

6 There is evidence that in severe chronic anxiety states adrenal cortex responsivity may be seriously disturbed and it is improbable in spite of specific treatments that the clinical condition will improve much as long as the adrenal cortex response remains poor

7 Since we understand clinically more about anxiety states than any other psychiatric disorder the evidence that adrenal cortex function is seriously disturbed in these conditions should invite particular study

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Suprarenal Cortex Activity in the Endocrine Equilibrium of Humans

by

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ONE of the most important characteristics of living organisms is their ability to main tain internal equilibrium The more complex the organism the more difficult does it become to maintain this equilibrium disturbance of which leads first of all to disease and then if sufficiently severe to death

The ductless glands are among the most important regulators of equilibrium Dis turbance of the hormone balance always leads to pathological changes of which but a few are sufficiently circumscribed to permit of accurate analysis and descrip tion In the present state of our knowledge it is possible to deal only with major endocrine imbalance The endocrine equilibrium depends first upon the hormone demands of the body under different conditions second upon the ability of the body to produce its hormones and third upon the interaction of the various hormones

HORMONE DEMAND AND HORMONE PRODUCTION

Our general ideas about hormone demand and production are based on animal experiments, and are still somewhat crude But even in animal experiments one can see that the relation between supply and demand does not always follow the same pattern neither in the same species under different circumstances nor in different species under the same circumstances If we compare for example the thyroid activity (Fig 1) of hedgehogs and squirrels in winter and summer we see that in winter the activity of the squirrel thyroid is at its highest while that of the hedgehog is at its lowest The decisive difference is that the hedgehog is hibernating and does not require increased oxidation owing to the cold weather, whereas the squirrel does The one to four day old rat which needs the animal warmth of the mother shows as can be measured by the phosphorylization of the adrenal an enormous increase in its activity as soon as it is removed from the mother and kept at room temperature for even half an hour This animal reacts to a temperature difference of about 16 C (between 36° and 20 C) whereas the adrenal of an animal about fourteen days old no longer reacts to such small difference in temperature (Reiss and Halkerston 1950)

Many similar differences can be found in human endocrinology during matura tion particularly in old age when the demands for the hormonal regulator seem to decrease and in spite of there being no clinical disturbance noted a post mortem examination will occasionally reveal the partial atrophy or even complete absence

of functioning adrenal or pituitary tissue Constitutional type also decides the extent of the hormone demand under certain environmental or stress conditions, and there fore also, the severity of the disease manifestation consequent upon functional dis turbances of the glands One of the most important facts however in determining the hormone demand, is the relative sensitivity to hormone of the body tissues Disturbed sensitivity to a special hormone can probably, in many instances, force the gland to ever increasing production and so result in the development of hyper functional states

INTERPLAY OF PITUITARY ANTERIOR LOBE HORMONES

Experimental investigation of the different anterior lobe hormones has shown the *existence of antagonisms between the individual hormones ACTH for instance is* antagonistic to growth hormone TSH and gonadotrophic hormone—and TSH is antagonistic to growth hormone and gonadotrophic hormone Considering that the anterior lobe hormones are continuously produced, TSH and ACTH in particular in accordance with demands made by the environment, it can be guessed how com*plicated is the interplay between the hormones Small causes can disturb the equili* brium and this disturbance soon leads to disease

Investigations carried out in our laboratories about the influence of electro shock on the pituitary anterior lobe of mental patients (Reiss, Hemphill Maggs Haigh Reiss 1951) show how varied the hormone response can be to the same stimulus Hyperthyrotic patients often show a decrease and hypothyrotic patients an increase in their thyroid activity after ECT Some patients show an increase in the ACTH content of the blood others a decrease after ECT (Reiss Hemphill, Halkerston Badrick 1952), some show an increase in their corticosteroid or ketosteroid excre tion while others do not

One of the main drawbacks in endocrine research is that *most investigators restrict* themselves to the study of a single gland or even one special function of the gland, and arrive at conclusions which however prove untenable when the total function of the gland or the role played by the gland in the total equilibrium is considered In this connection Selye s theory about diseases of adaptation must be mentioned *Owing to its simplicity this theory readily commends itself to the non specialist* but it is superficial and may become positively misleading if included in pathophysiological concepts which are to form a basis for clinical therapy

DISTURBED HORMONE EQUILIBRIA IN HUMAN PATHOLOGY

Investigation of different clinical disturbances and changes in the biochemical equili brium changes accompanying clinical deterioration and clinical improvement offer ample opportunities for study of disturbed hormone equilibria and the mechanisms of their regulation This is only rarely possible in an animal experiment, since it is very difficult to investigate animals for many days or months under standard condi tions in order to get a base line for the study of subsequent changes (spontaneous or induced) in the hormone equilibrium

One of the most interesting instances of disturbed equilibrium is seen in a great number of hallucinated acute schizophrenic patients Fig 2 shows an example of

the ketosteroid excretion of one normal subject and one hallucinated schizophrenic Both these examples are typical of a great number of such excretion curves investigated during the last twelve years It can be seen that the day to day, week to week, and even month to month variation of normal individuals does not exceed ±15 per cent A schizophrenic, on the other hand can show day-to-day variations of ±50 to ±200 per cent These wide variations are apparently an expression of a disturbed equilibrium of the adrenal cortex, which is the main producer of ketosteroids The equilibrium in the production of corticosteroids is disturbed in a similar way and it is feasible to assume and will later on be shown that this is the expression of a much more involved disturbance of the total hormone balance This disturbance is apparently connected with the mental state of the patient In Fig 3 is seen the ketosteroid excretion during different phases of treatment of such a patient During treatment with ACTH the upper limits of the excretion rates are increased in this patient but the fluctuations in the excretion rate are unchanged No improvement of the mental state of the patient is achieved Electro shock treament shows no therapeutic influence either and no change in the fluctuations The fluctuations are also seen at the beginning of the insulin treatment but become less and disappear finally when the insulin treatment is stopped This patient shows a complete mental remission The cessation of the fluctuation was taken as a sign of restoration of the hormone equilibrium Other patients who did not improve after insulin also showed unchanged fluctuations in the excretion rate after treatment Similar correlations between biochemical change and mental state can also be seen occasionally when other kinds of treatment are used Fig 4 shows again the treatment periods of a schizophrenic, who was treated first with testosterone injections No change in the fluctuations is seen during the two months of this treatment and only occasional slight mental improvement which however was never maintained The patient then received a testosterone implantation The fluctuations ceased and the patient showed a complete and lasting remission

More interesting are the disturbances in the equilibrium in the thyroid adrenal interrelation Fig 5 shows extreme cases of such disturbances ketosteroid and cortin excretion rates are considerably decreased thyroid activity is above the normal range Patient 1 an acute schizophrenic boy Patient 2 is a woman suffering from puerperal depression Repeated investigations of ketosteroid and cortin excretion rates showed how both started to rise spontaneously and some time afterwards both patients showed a spontaneous remission Re investigation of thyroid activity during the remission showed that it had decreased to within the normal range It may be added that the responsiveness of the pituitary adrenal system was disturbed at the start of the observation in both cases and considerably improved when re investigated at the time of the biochemical and mental improvement Such simultaneous changes can also take place the other way round Before admission patients may show increased ketosteroid excretion rate and decreased thyroid activity whereas after hospitalization their ketosteroid excretion rate starts to go down into the normal range while their thyroid activity rises to normal values Such changes are also accompanied by remission of their mental disease It has become the custom in our laboratory to investigate some patient groups from this viewpoint and the disturbed equilibrium in the adrenal thyroid interrelation and in case the interrelation is disturbed to wait for one to

two weeks to see whether any spontaneous changes occur If this is not the case, one studies what treatment can improve the hormone equilibrium Electro shock and also deep insulin therapy can occasionally do so but much more interesting are trials where such an improvement is achieved by less dramatic means, as is shown for instance in Fig 6 where ketosteroid and cortin excretion rose and remained steady at normal level after testosterone treatment, while the originally increased thyroid activity became normalized So also did the originally disturbed adrenal cortex responsiveness and the patient recovered from severe depression

It would be tempting to include in the description of the adrenocortical role in the hormone equilibrium another most important counter actor, the gonads which are functionally antagonists, in some directions, to both the thyroid and the adrenal cortex The atrophy of the testicles seen in schizophrenia the deterioration in mental condition in relation to different stages of the menstrual cycle, and the disturbance of this cycle accompanying disturbances of the thyroid activity already give us many hints about their important role in the hormone equilibrium But there are, at present not yet enough reliable and in particular measurable data known

The antagonism between thyrotrophic and adrenal corticotrophic hormone has been known to us for many years (Reiss and Peter, 1938), and has only lately re ceived more attention, since ACTH has come into the limelight Its investigation should become very productive in a great many not only psychiatric clinical syn dromes There is no need to mention here the well known symptomatology of Addison s disease and the well known dilemma a clinician has sometimes to over come, when he sees states of emaciation which over activity of the thyroid and the differential diagnosis of adrenal cortical under function has to be faced Too rarely is attention paid to the involvement of the adrenal cortex activity in cases of thyro toxicosis

I have discussed in this paper a few simple examples of not uncommon changes in hormone equilibria This has been done deliberately in order to counteract the one way traffic in thought and research progress which was started by Selye s concept of the so called general adaptation syndrome, and was subsequently intensified by the popularization of ACTH Many valuable developments in research are in danger if, relating too many symptoms to the adrenal cortex alone we forget that it plays but a proportionally modest role inside the total hormone equilibrium of the body

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THYROIDS

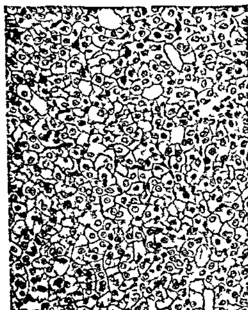


Winter



Summer

HEDGEHOG



Winter



Summer

SQUIRREL

Figure 1 Thyroids of hedgehogs and squirrels investigated in winter or summer time

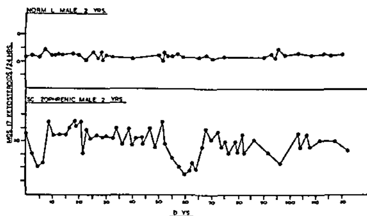


Figure 2 Changes in the 24 hr/17 ketosteroid excretion rate of a normal male control and of an acute male schizophrenic

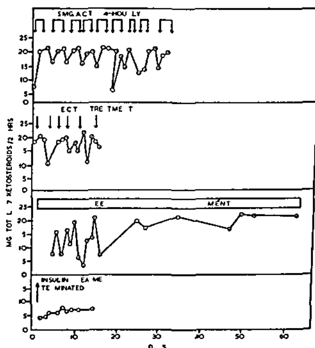


Figure 3 Comparison of the influence of different treatments on the ketosteroid excretion rate of an acute schizophrenic male patient (1) ACTH treatment (2) ECT (3) Deep insulin

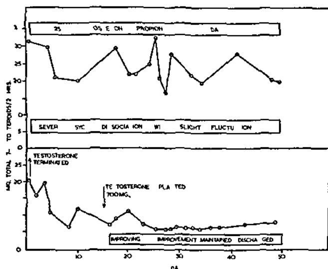


Figure 4 Comparison of the influence of testosterone injections and a testosterone implant on the 17 ketosteroid excretion rate of an acute schizophrenic male patient

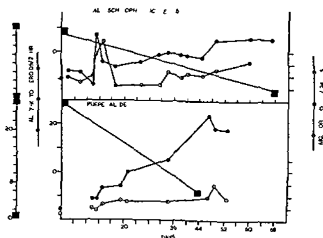


Figure 5 Spontaneous changes in the 17 ketosteroid excretion rate the corticosteroid excretion rate and the thyroid activity during spontaneous improvement of two mental patients

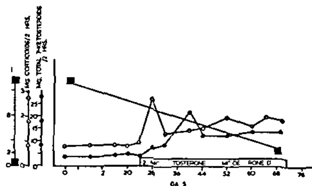


Figure 6 Changes in the 17 ketosteroid/24 hr excretion rate corticoid/24 hr excretion rate and thyroid activity in a depressed patient who recovered after testosterone and oestrone treatment

Discussion

ON PAPERS BY (1) HOAGLAND, (2) HEMPHILL, (3) REISS

Chairman A V Neale

A Faurbye I am reluctant to comment on three such excellent papers There is no doubt that the adrenal cortical hormone does have an effect on the brain and thus on the psyche I am looking forward with much interest to hearing the final results of adrenalectomy in schizophrenics

I agree with Dr Reiss that it is important to view the entire hormone equilibrium of the patient and not just to study one gland

I think that electroconvulsant therapy has a direct effect on the brain and that this direct effect is more important than the indirect one through the hypothalamus pituitary suprarenals etc The direct effect of ECT on the brain is well shown by the loss of memory which patients experience This may be very temporary or persist for months or years Also it is sometimes possible to cure a psychotic patient by ECT without getting any response from the suprarenal cortex In this I think Dr Hemphill will agree with me

A Segaloff I am disturbed by much that has been said today It seems to me we are erring in interpreting some of the data on adrenal function with relation to specific disorders We are applying multiple tests to patients suffering from certain conditions finding abnormal results and then interpreting them as being specific for that disease However similar findings with regard to adrenal physiology have been seen in other chronic illnesses It appears to me that the essential question is whether or not we have simply proven by these findings that schizophrenia is a chronic illness In other words is it fair to compare these results to those obtained in normal healthy individuals or must we use as controls patients suffering from some other chronic illness? Thus basically the problem is a matter of what constitutes an adequate control group of such studies

A V Neale The EEG of Addisonian patients is restored to normal by cortisone, do you know whether it is merely the water retention that is causing this?

The reduced cerebral blood flow in these patients is also restored to normal by cortisone is it merely the increased blood pressure that brings about this change?

H Hoagland I am unable to answer Professor Neale s question and know of no data about Addisonian patients along these lines

Levine has done work on the blood flow in the mesoappendix of rats in which he has shown that cortisone acts on the arterioles and that norepinephrine acts synergistically with it to produce constriction In animals adrenalectomy has been reported to increase capillary permeability to produce bradycardia and to lower systemic blood pressure

R E Hemphill The question of performing adrenalectomy on psychiatric cases raises very serious issues, much wider and with a greater degree of responsibility even than with psycho surgery I should like to ask Dr Hoagland some practical questions about the procedure adopted

1 What is the method of selection of the patients for the operation? Is it on purely psychiatric grounds or as the result of chemical hormone analysis?

2 What were their ages, and was this taken into consideration?

3 Were they males or females?

4 Are the relatives fully informed of all the pros and cons, and if so do they really accept responsibility for the eventual care of patients in the event of improvement and discharge?

I know it is much too early to offer any figures about success or failure but I should have thought that as patients cannot be regarded as experimental subjects under any conditions at all when they are not capable of giving their full and free consent, the prognostic value of the operation would have to be taken into consideration when obtaining consent

H Hoagland The patients for bilateral adrenalectomy were selected by the psychiatrists They were chronic schizophrenics hospitalized for an average of ten years Two of the patients had carcinoma with metastases One woman had carcinoma of the breast and one man had carcinoma of the prostate The four other patients were non cancerous schizophrenics who had made good premorbid adjustments I regret to say that none of the Chicago patients were selected on the basis of adrenal function studies Of course an important determining factor was the ability to obtain enlightened consent from families The patients ranged in age from thirty four to sixty five years, three were women and three were men The relatives were fully informed of the nature of the operation and its likelihood of success

There are other criteria besides studies of steroid metabolism which influenced our decision to perform bilateral adrenalectomy on schizophrenic patients Prolonged heavy dosage with cortisone and ACTH sometimes produce a psychosis in patients hitherto non psychotic, and Cushing s disease and suprarenal neoplasm are frequently accompanied by psychosis Mr Broster's cases in which the removal of one hyperplastic suprarenal has relieved psychosis diagnosed as schizophrenia should also be borne in mind Our metabolic studies indicate a derangement of steroid metabolism in schizophrenia Removal of such adrenals and the administration of suitable replacement therapy has been our objective

M Reiss In reply to Dr Segaloff re controls The normal range of twenty four hours urinary ketosteroid excretion rate is well known About the normal range of thyroid activity see Reiss, Haigh Hemphill *J Endocrinology* 8 *1* 1952

I should like to repeat that it is vital to get some criteria on which to control the dosage of ACTH and cortisone

L. R Broster I have been fascinated by this discussion I have received criticism in the past for my surgical work on the suprarenals, but I took the long view and now most of the criticism has been silenced by recent work Nobody knew very much about the suprarenals when I started operating on them, but now the suprarenal

function is being examined in detail and Dr Hoagland has made a good start in using the results

The case he referred to was a girl whose suprarenal I removed some ten years ago for a psychotic condition She now works in the Post Office in London and has been quite well ever since except for a recrudescence of her symptoms at the menopause almost leading to her certification but this has now settled down again (Allen *et al* *B M J* 1 1220 1939)

I was fascinated by Dr Vogt s paper and feel we must tackle these psycho somatic conditions The next twenty years should give us the answer to many of the suprarenal problems facing us today We seem to be reaching the hypothalamus in stages

A Survey of Tissue Responses to ACTH and Cortisone

by

G R CAMERON

Department of Morbid Anatomy University College Hospital Medical School London

It is my task to present to you the facts which have accumulated about tissue responses to cortisone and ACTH They are numerous often disconnected apparently unrelated and sometimes ill defined but I believe that already some sort of guiding principles are emerging from which fundamental conclusions can be drawn and future investigations planned I would ask you to keep in mind the reservation that I am approaching this discussion as a pathologist, one who devotes his time to the study of reactions of living material to injury in the broadest sense Such an approach has its limitations though these perhaps, are inherent in all biological investigation Sooner or later we are thrown back upon the units of life for the answer to our questions this has certainly proved to be the case with my own inquiry

I ACUTE INFLAMMATION AND ALLIED CONDITIONS

Cortisone in therapeutic doses diminishes the active inflammatory responses in human subjects with rheumatoid arthritis (Hench *et al* 1949 1950 and many others) as a result oedema decreases in and may disappear from joints muscles and other articular tissues with lessening of tenderness stiffness and pain Serial biopsies from patients under treatment show diminution of inflammatory exudate and cellular infiltration This response takes a few days to develop persisting or even increasing with treatment but relapsing when treatment is stopped or the dose of cortisone falls below a certain level (Cameron 1951)

Rheumatoid nodules become smaller or disappear, enlarged lymph nodes decrease in size the acute lesions of many collagen diseases such as dermatomyositis lupus erythematosus and periarteritis nodosa show rather slow healing (Shick *et al* 1950 Drury *et al* 1951) A decrease in the cellular infiltration of the affected areas of the liver in infective hepatitis after cortisone treatment has been recorded by Hanger and Collins (1950) So too a variety of severe hypersensitivity reactions may be suppressed by ACTH or cortisone (Bordley *et al* 1949 and many others)

Equally clear evidence of diminished inflammatory responses comes from experimental studies Fractures in cortisone animals exhibit much less fluid exudation and fibrin than in untreated animals (Blunt *et al* 1950 Sissons and Hadfield 1951)

The traumatic inflammation that accompanies the healing process of skin autografts in rabbits is suppressed when cortisone is given in large doses (Billingham *et al*, 1951) Selye (1949a) demonstrated considerable reduction by cortisone of periarticular inflammation induced in rats by localized formalin injections but other workers have not been able to confirm his results (Parkes and Wrigley, 1951) The exudate and fibrin of skin wounds is greatly lessened by the exhibition of cortisone (Spain *et al*, 1950*a*, *b*, Michael and Whorton 1951, Coste *et al*, 1951) as is also the case with allergic skin responses (Selye 1949*b*, Long and Miles 1950 Derbes *et al*, 1950, Harris and Harris, 1950, Bangham—unpublished) Shwartzman *et al* (1950, see also Shwartzman, 1950) showed that the local reaction produced in rabbits by injecting intradermally meningococcal culture filtrate followed twenty four hours later by the intravenous injection of a large provocative dose of the material can be inhibited in most cases by a previous intramuscular injection of cortisone Confirmatory results in a variety of allied inflammations have been obtained by Dougherty and Schneebeli (1950) and by Armstrong and Irons (1951)

The elegant experiments of Biegel (1951) and of Ashton and Cook (1951) clearly demonstrate that cortisone inhibits the formation of plasmoid aqueous and fibrinous collections in the injured anterior chamber of the rabbit's eye and in the damaged cornea Jarvinen (1951) has shown that the sensitivity of the human skin to radiant energy can be much reduced by cortisone, the minimal exposure required to give an observable reaction being approximately doubled under such circumstances Vascular dilatation is reduced, and blistering is less prominent Jarvinen therefore suggests that cortisone suppresses some kind of protective mechanism Obviously the question of vascular response needs further study In all of these experiments the outstanding result has been the reduction of oedema and therefore of fibrin formation and of cellular exudate both polymorphonuclear leukocytes, macrophages and lymphocytes The initial vascular hyperaemia too, has been described as diminished by some observers

2 PERMEABILITY EFFECTS

Since the mechanism of inflammation is so closely bound up with the problem of permeability of tissues and blood vessels it is not surprising to find that a search has been commenced for an influence of cortisone on permeability Some success has already been achieved in the following fields

A Spreading factor

It has been shown that the increase of permeability of connective tissue and blood vessels induced by hyaluronidase (spreading factor) whereby the intradermal spread of India ink and the leakage of dyes such as *T 1824* or trypan blue from the blood into the tissues is speeded up can be inhibited or suppressed by local or systemic cortisone therapy (Opsahl 1949, Seifter *et al* 1949, Shuman and Finestone 1950, Benditt *et al* 1950) Since cortisone has no action on hyaluronidase *in vitro* it must surely exert its effect on the tissue and endothelial cells or their ground substance or possibly by means of some indirect reaction but we know nothing about these possibilities as yet

B Vascular permeability factors

Lessening of inflammatory exudate points indirectly to an alteration of the capillary permeability for plasma proteins and other colloids This view is supported by an observation that we owe to Armstrong and Irons (1951) who found that ACTH greatly lowered the protein concentration of vesicle fluid from a patient with sclero derma

Up to the present, evidence that cortisone or ACTH may modify the capillary permeability of healthy animals is wanting but there is reason to believe that in creased permeability may be reduced and brought back to a normal level through the action of cortisone This may well be the explanation in part of the therapeutic action of the hormone since a great deal of the improvement is due to subsidence of inflam matory oedema in the affected regions The action of cortisone on the permeability increase, as shown by leakage of large molecular dyes from capillaries in the presence of hyaluronida e has already been mentioned Studies with histamine the agent which is largely responsible for exudation of fluid at sites of inflammation show that in rabbits large doses of cortisone can reduce capillary permeability (Bangham 1951) Similar results have been demonstrated again by Bangham with the leukotactic peptides of Spector (1951) these too, are liberated when tissues are injured and provoke several of the inflammatory responses The anti histamine action of cortisone has not been demonstrated in human subjects given therapeutic doses of the hormone but some observations suggest that the abnormal histamine sensitivity of the asth matic is lowered by ACTH

C Capillary permeability in the eye

The transfer of fluorescein across the blood aqueous barrier of the eye is a measure of the capillary permeability rather than of the rate of blood flow (Duke Elder and Ashton 1951) it is therefore significant that cortisone leads to no alteration in fluorescein transfer in the rabbit or human normal eye (Leopold *et al* 1951 Cook and MacDonald 1951) In contrast are the findings of the latter workers in the in flamed eye where the capillary permeability is greatly increased Administration of cortisone either locally by the instillation of drops or subconjunctival injection or parenterally, reduces the permeability to the normal level True the effect is a tran sient one and the eye soon returns to its abnormal state but there can be no question that cortisone has as it were come between the agent responsible for the inflamma tory alteration in the vessels and the capillaries

3 REPAIR

Prior to the isolation of cortisone it was suspected that the adrenal cortex produces hormones which influence adversely the healing of wounds Kosdoba (1934) showed that prolongation of healing occurred in animals with homotransplants of adrenal tissue or when the adrenals were stimulated observations which suggest that increase in the amount of circulating adrenal hormones is responsible for this phenomenon Ragan Grokvest and Boots (1949) found poor wound healing in patients with hyperadrenalism and slow repair of open wounds incised abscesses and biopsy sites in subjects under treatment with ACTH Creditor *et al* (1950) Plotz *et al* (1950*a* and

b), Behrman and Goodman (1950), Videback *et al* (1950) have also demonstrated in man the suppression of granulation tissue in skin wounds by ACTH and cortisone

Experimental studies have, for the most part, afforded confirmation of human experience Ragan *et al* (1949) depressed the formation of granulation tissue in rabbits' ears with ACTH, Baker and Whitaker (1950), Shapiro *et al* (1951) and Bangham (1951) in the rat, Spain *et al* (1950*a*) in mice Plotz *et al* (1950*b*), Creditor *et al* (1950), Ragan *et al* (1950) Stinchfield (1950) and Bangham (1951) in the rabbit have obtained similar results with large doses of cortisone * Species differences are pronounced, the rabbit responding best the guinea pig least, to these hormones In successful instances granulation tissue shows fewer and smaller blood vessels and there is sparse fibroblastic proliferation In other words, the inhibition is quantitative not qualitative and so far as can be determined by histological methods reparative tissue is normal in structure and function though greatly diminished in amount

Similar results have been recorded with the healing of fractures in cortisone animals (Blunt *et al*, 1950, Plotz *et al*, 1950*b*, Sissons and Hadfield 1951) Callus forms in less amounts and union is delayed Retarded healing has also been described in wounds of the stomach (Plotz *et al*, 1950*b*) in the primary healing of pinch grafts and skin autografts in rabbits (Billingham Krohn and Medawar, 1951), following the injection of turpentine in rats (Taubenhaus and Amromin, 1950) and in the peritoneal reaction in the rat to talc (Ducommun and Mach, 1950), and to finely powdered quartz (Magarey and Gough 1952) The latter workers found clear evidence of inhibited fibrosis in the mouse and rat temporary inhibition in the rabbit but no inhibition in the guinea pig Less fibrous tissue formation has been claimed in the spleen and lungs of rats infected with *Coccidioides immitis* and treated with cortisone (Cavallero and Sala, 1951) and in the liver of rats injected repeatedly with carbon tetrachloride (Aterman, 1950 Cavallero *et al* 1951)

Some interesting results have also been recorded in the eye Inhibited wound healing is found in the cornea of rabbits the effect depending on the concentration of cortisone in the animal s body fluids and on the route and period of administration (Leopold *et al*, 1951, Ashton and Cook, 1951) These experiments are of especial interest since corneal healing after such wounds may go on without the intervention of new blood vessels so that the delay with cortisone cannot be attributed to vascular interference (Duke Elder and Ashton, 1951) On the other hand, eye experiments show quite clearly that cortisone can exert considerable inhibitory influence on the formation of new vessels Jones and Meyer (1950) injected sodium hydroxide into the cornea of rabbits and found that vascular invasion was subsequently depressed when the animals were injected subconjunctivally with cortisone Confirmatory results have been obtained by Lister and Greaves (1951) and Ashton, Cook and Langham (1951) The cause of the depression of vessel invasion has not yet been discovered though the demonstration by Ashton and Cook (1951) of cortisone inhibition of the healing of the endothelial lining of Descemet s membrane suggests a direct action on endothelial cells

All of these observations, both in man and experimental animals, combine to show that cortisone in large doses retards the healing of inflammatory lesions whether

* Dr Robert Buck of my department has recently shown that fibrous tissue regeneration in tendon is inhibited quantitatively in the rat by large doses of cortisone

they be aseptic or infective traumatic or chemical Both endothelial cells and fibrous tissue cells form in smaller numbers though in normal pattern so that the difference in repair in cortisone and control animals is quantitative only

Information about epithelial regeneration so far has been confined to wound healing and is contradictory Most observers agree that epithelium grows over the surface of a wound in normal fashion even though there be substantial retardation of granulation tissue (Spain *et al*, 1950*a*—mice Creditor *et al*, 1950—rabbits Ragan *et al*, 1950—rabbits Bangham 1951—rabbits rats, Baxter *et al* 1951—man Ashton and Cook 1951—rabbits corneae Lister and Greaves 1951—rabbits corneae) * Diminished epithelialization of wounds has been claimed by Howes *et al* (1950) Baxter *et al* (1951) and Leopold *et al* (1951) We badly need information about the action of cortisone on various kinds of epithelial regeneration as in the claim of Green and Ghadially (1951) that cortisone is a powerful inhibitor of mitosis in the prophase in the epidermis of the adult male mouse

4 ACTION ON CELLS

Some of the facts that have already been mentioned suggest that cortisone may exert a direct action on cells This seems to be the case with fibrous tissue cells especially when they are participating in repair and endothelial cells in the early as well as the reparative stages of inflammation With all such observations however we cannot exclude the possibility of an indirect action of cortisone We are in urgent need of investigations into the direct action of the hormone on cells but these are difficult to carry out because of the physical and chemical properties of cortisone Tissue culture methods are being employed but results again are contradictory Inhibition by large doses of cortisone of the growth of fibroblasts and macrophages *in vitro* is claimed by Barber and Delauney (1951) Cornman (1951) and Holden *et al* (1951) Steen (1951) however finds no such inhibitory effect in concentrations of cortisone comparable to and even twenty five times greater than the usual therapeutic levels Growth was inhibited by doses far in excess of therapeutic concentration but other factors most likely were responsible Clearly the subject needs further critical study

More suggestive evidence comes from experiments on animals There is reason to believe for instance that macrophage structure may be altered and phagocytic activity inhibited (Gordon and Katsh 1949) that lymphoid cells within the great lymphocytic depots—thymus spleen and lymph nodes—are destroyed through the action of cortisone and ACTH (Ingle *et al* 1938 Wells and Kendall 1940 Heilman 1945 Ingle 1950 Stebbins 1950 Wilkins *et al* 1950, Winter *et al* 1950 Antopol *et al* 1951), that eosinophilic leukocytes are depleted and that prolonged administration of the hormones leads to withering of adrenal cortical cells as the result of disuse atrophy Neoplastic lymphoid tissue is also destroyed by cortisone (Heilman and Kendall 1944 Pearson *et al* 1949 1950 Stoerk 1950) We have mentioned the observation of Green and Ghadially that mitosis in epidermal cells of male mice can be inhibited by cortisone Billingham *et al* in confirming this observation suggest that inhibition is due to the action of cortisone on blood vessels of the skin

* Regeneration of tracheal epithelium in the rat has been shown by Dr D Wilhelm in my department to be unaffected by cortisone

Linked with cell disturbance may be the inhibition of body growth with which is associated increased protein breakdown, reflected in the increase of urinary non protein nitrogen (Wells and Kendall, 1940, Marx *et al*, 1943, Baker *et al*, 1948, Hench *et al*, 1949, Ingle, 1950) Browne (1951) suggests that cortisone and other corticosteroids simply make the body proteins more easily mobilized Storage of glycogen in the liver cells is also a prominent side effect of cortisone, while fat absorption and depot fat storage may be stimulated in certain localities The increased excretion of potassium associated with cortisone or ACTH administration suggests a cellular disturbance (Sprague *et al*, 1950) resulting in loss of potassium Some evidence from studies of muscle electrolytes of patients is in favour of this view (Kepler *et al*, 1948, Eliel *et al*, 1950)

Finally, the action of cortisone on antibody production and resistance may, for the time being, be associated with cellular factors A little evidence has been collected that antibody production in acute infectious diseases is inhibited or prevented by cortisone in massive doses (Germuth and Ottinger, 1950, Glaser *et al*, 1950, Bjorneboe, 1951) but again the evidence is conflicting It has also been established that cortisone accelerates or intensifies the development of chronic diseases such as experimental pneumococcal or streptococcal pneumonia (Glaser *et al*, 1950), sub acute streptococcal infections (Lewis *et al*, 1951), tuberculosis (D'Arcy Hart and Rees, 1950, Spain and Molomut, 1950, Block *et al*, 1951), brucellosis (Abernethy, 1951), syphilis (Wolf *et al*, 1951), trypanosomiasis (Soffer *et al*, 1950) and experimental malaria (Schmidt and Squires 1951) We have already mentioned the depressive action of cortisone on a variety of allergic responses, themselves, in turn, cellular in origin In some of these examples altered resistance is clearly associated with depletion of cells such as lymphocytes and macrophages well known to be devoted to the protection of the organism against infection * Here, again, is a fertile subject for investigation

DISCUSSION

From this mass of interesting but often disconnected facts we may select certain guiding principles for further thought and research

1 Cortisone inhibits the response of tissues to injury as a result we find at the affected site

- A Lessened vascular hyperaemia
- B Less exudation of fluid i e less oedema
- C Less fibrin deposition
- D Diminished cellular migration and infiltration

2 Cortisone retards the early stages of repair

- A By inhibiting fibroblast formation
- B By reducing the formation of new capillaries and lymphatics
- C By depressing phagocytic activity and thus delaying the removal of by products of tissue damage
- D By destruction of lymphoid cells and eosinophils
- E Possibly by inhibiting mitosis in certain reparative cells

* We have noticed that our rats sometimes develop bartonellosis during cortisone treatment probably because of inhibition of lymphoid and splenic function It is well known that splenectomy often leads to a flare up of bartonellosis in rats

3 Cortisone in large doses lowers the resistance of the organism
 A Through depression of antibody production
 B By depression of hypersensitivity reactions
 C By a general depression of resistance to many acute and chronic diseases

4 Cortisone makes cells especially endothelium less sensitive to agents which normally increase permeability to proteins, especially spreading factors, histamine and leukotactic peptides

5 Cortisone inhibits growth increases protein breakdown increases storage of carbohydrate in liver cells and encourages fat absorption and storage

It should be emphasized that many of these effects are the outcome of large doses of cortisone, but that others are encountered with therapeutic doses Taking into consideration the well known variation in response to hormones drugs and poisons in animal species it seems reasonable to accept the above list for guidance in a study of the biological potentialities of cortisone Clinical studies too indicate that the response needs a few days to develop and that relapse with recurrence of symptoms is inevitable when treatment is stopped or the dose of the hormone falls below a certain critical level We are thus led to the conclusion that cortisone inhibits certain tissue responses *for a time* especially those caused by injuries of various kinds and particularly the fundamental disturbances common to all types of injury *Cortisone damps down these responses, it does not modify the primary cause of the pathological reaction* We must therefore turn to the living elements, the cells and their derivatives and components, if progress in the investigation of cortisone is to be made The surface action of cortisone must be studied since permeability is in the first instance a surface phenomenon Such effects as decreased leakage of plasma from capillaries and transfer of inorganic ions and water between extracellular and intracellular phases (Kendall 1941), lessened fibrin production inhibition of inflammatory responses interference with phagocytosis growth and lowering of resistance to various infections may well reflect a common disturbance of surface activities of cells brought about by concentration of cortisone in the lipoid moiety of the cell membrane

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Steroid Hormones and Skin Grafting

by

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As a rule, skin cannot be grafted from one human being to another or from one animal to another Within a relatively short time the epidermis of the graft is destroyed and although the more inert tissues of the dermis may continue for a little longer the whole graft finally disappears Orthotopic grafts survive only in the following circumstances

1 Grafts between monozygotic twins

2 Grafts between members of strains so highly inbred as to be virtually monozygotic

3 Grafts between dizygotic twin calves The analysis of the blood groups of such twins shows that haematopoietic cells which persist into adult life may be transferred from one embryo to the other presumably via the placental circulations which are usually conjoined Such exchanges of tissue might well desensitize the recipient and enable it to tolerate a graft of foreign skin

A close study of rabbits [Medawar (1944 1945)] has shown that for the first few days after grafting homografts behave in the same way as autografts They heal as well acquire a new blood supply and start on a similar period of intense mitotic activity But between the sixth and ninth day after grafting they become very inflamed They swell, harden and become discoloured The surface epithelium can be scraped off as a wet paste, the edges lift away and the grafts finish as hard mushrooming scabs connected to the graft bed by fibrous stalks

Microscopic examination shows that the process of decay is associated with oedema of the tissues tagnation of the circulation and cellular mainly lymphocytic infiltration The walls of the blood and lymphatic vessels lose their integrity and become disrupted

There is a considerable amount of evidence to suggest that this degenerative process is the outward sign of a systemic immunity response which develops to the homograft tissue Attempts to isolate the presumed antibody or to demonstrate any effects of a medium containing immune serum on tissue cultures of skin have however so far failed

Cortisone has a powerful influence on the tissues which are probably responsible for immunity reactions and has been shown to affect the building up of immunity to different types of antigen We* have accordingly studied the influence of cortisone on the homograft reaction on the assumption that it too is a manifestation of an acquired immunity (Billingham Krohn and Medawar 1951 *a b*)

* The experiments reported in this paper represent the combined work of R E Billingham P L Krohn and P B Medawar

METHOD

In the first series of experiments a suspension of cortisone was administered systemically by subcutaneous injection [10 mg daily of cortisone acetate (Merck)] In later experiments cortisone was applied locally The general plan of experiments was the same throughout

Adult rabbits of either sex were used, the donor recipient pair being of different breeds The aim of this selection was to ensure the maximum genetic diversity and in this way (*a*) to intensify the homograft reaction, and (*b*) to make the test of cortisone as severe as possible

The grafts were full thickness pinch grafts about 9 mm in diameter, taken from the dorsum of the ear They were transplanted—in open style, autografts and homografts alternating in chessboard pattern—on to a rectangular raw area on the side of the chest This area was prepared by stripping off the skin, the panniculus carnosus muscle being left intact The technique has been described in detail by Billingham and Medawar (1951)

The dressings were removed for inspection of the grafted area on the sixth day after operation and subsequently at three day intervals An accurate assessment of the time at which a graft has completely broken down cannot be made on the basis of naked eye appearances alone Biopsy specimens were therefore removed at three day intervals for histological examination

THE EFFECTS OF TREATMENT WITH CORTISONE

Operation areas

The wound normally heals by spread of epithelium outwards from the autografts, by ingrowth from the edges of the wound in which thick granulation tissue develops and by a generalized contraction of the surrounding tissues The process can be estimated from measurements of the dimensions of the wound

Cortisone inhibits all these normal changes Granulation tissue develops very sparsely, the finest vessels in the panniculus remain visible, and for many days the raw areas appear as if only recently prepared Contraction is slight and the dimensions of the raw area decrease very slowly Normally the sites on the ears from which grafts are removed scab over, and are soon re epithelialized Only a very thin weepy coagulum was found in the cortisone treated animals

Autografts

The grafts healed much more weakly than normal grafts and they were much less vascularized The burst of mitotic activity that usually occurs about six days after grafting was very reduced, and the outward spread of epithelium was diminished This inhibition of mitosis could presumably be simply a consequence of the inadequate development of a new capillary blood supply The possibility of a direct anti mitotic effect of cortisone cannot however be excluded The growth of new hairs and re pigmentation was also delayed

Homografts

The effect of the treatment was to convert the normal acute progressive reaction into a slow chronic process The inflammation was moderated and the lymphocytic

infiltration was curbed In general, the result was to diminish the intensity of the reaction but not to change its character The most important effect was that the grafts judged by histological criteria, survived three to four times as long as usual

THE EFFECT OF LOCALLY APPLIED CORTISONE

It was clear from the above results that cortisone administered systemically can prolong the life of skin homografts though the grafts do not survive indefinitely A second series of experiments showed that local application of cortisone is also effective Five mg of the ordinary suspension of cortisone acetate was smeared over the raw area with a glass rod at the time of operation and every third day afterwards It is more than likely that this is not the most satisfactory way of applying cortisone locally but even so the treatment doubled the life of the grafts and had the expected effects on mitosis, build up of granulation tissue and wound healing A similar dosage of cortisone given either systemically or by local application to a second raw area prepared on the opposite side of the body was without effect on the homografts, indicating that the influence of cortisone was essentially local

THE MECHANISM OF THE ACTION OF CORTISONE

The effect of cortisone might be due to one or more of several factors (1) interference with the local tissue reactions at the site of interaction of graft antigen and antibody or (2) to diminished ability of the grafts to elicit an immunity reaction or (3) to interference with the rabbit s normal production of antibodies

If a rabbit that is not treated with cortisone is given a second set of grafts from the same donor, the behaviour of the grafts is modified, and they break down more rapidly than the first set The recipient has already developed an immunity and the reaction to the new set of homografts begins without any delay Cortisone given either systemically or locally to such pre immunized animals failed to prolong the survival of the grafts

In another series of experiments a set of homografts was grafted to each side of a rabbit and cortisone applied locally to one side only The grafts treated with cortisone did not live as long as they would have done had all the homografts been treated with cortisone and the difference between the survival time of grafts on the two sides was small This observation suggests that the immunity that was provoked by the untreated grafts led to the destruction of the homografts on the cortisone treated side

From both these experiments therefore it would appear that cortisone does not interfere with the antibody antigen reaction The effectiveness of cortisone applied locally to a first set of grafts as described earlier indicates that what might be called the afferent side of an immunity reflex arc is affected It is not known whether treatment with cortisone diminishes the supply of antigenic material from the grafts to the site of antibody formation But the decreased vascularity and diminished cellular proliferation that are induced by the local or systemic administration of cortisone might have this effect

There is ample evidence that the level of anti bacterial antibodies falls rapidly if cortisone is administered Since the gradual removal of passively administered

antibody is not accelerated by cortisone (Fischel, Stoerk and Bjrneboe, 1951), it is assumed that the synthesis of antibody is impeded Whether this is true of the immune response to homograft tissue is not known If cortisone can have such an effect, prolonged treatment of an animal that had already become immune in response to a first set of grafts would provide time for the removal in the ordinary way of existing antibody, and at the same time prevent the formation of any fresh antibody In such circumstances the usual reaction to a second set of grafts would not occur, and provided the treatment was continued the grafts might survive as long as first set grafts An experiment on these lines is being carried out at present, cortisone being given to rabbits for twenty days before the second set of grafts are transplanted The results are not clear cut but they seem to suggest that the cus tomary reaction to the second set of grafts may be impeded If this result should be confirmed it would follow that the effect of treatment with cortisone depends both on its interference with the afferent side of the immunity process and on antibody production, but not on the local results of antibody antigen interaction

THE EFFECT OF ACTH AND OTHER HORMONES ON THE SURVIVAL OF HOMOGRAFTS

Several attempts have been made to see if treatment with ACTH would reproduce the effects of treatment with cortisone Doses of up to 10 mg (equivalent LAIA) three times daily, have been given subcutaneously without producing any consistent increase in survival time of the grafts

It seemed possible that treatment of rabbits with ACTH might stimulate the pro duction of adrenocortical steroids which could antagonize the effect of cortisone Accordingly we have carried out experiments in which oestradiol dipropionate, testosterone propionate, progesterone or deoxycorticosterone acetate have been given systemically, either independently or in combination with cortisone There was no evidence that any of the hormones by themselves influence the homograft reaction nor did their administration concomitantly with cortisone consistently prevent cortisone s effect either on the grafts or graft bed The atrophy of the adrenal cortex and hypertrophy of the liver that follow treatment with cortisone were also unaffected An unexpectedly short survival time (in only one rabbit of several given DCA and cortisone) may possibly indicate that there is some antagonism between these two hormones

DISCUSSION

A number of American workers [e g Weisman Quinby, Wight and Cannon (1951)] have been unable to demonstrate that the reaction to skin homografts can be inhibited by cortisone or ACTH Morgan (1951) working on rabbits and Cannon and Longmire (1952) using chicks have on the other hand reported findings similar to our own In attempting to account for the discrepancies in the results of different workers, it is apparent that most negative findings relate to experiments in which guinea pigs were given ACTH The skin grafting techniques have also been different Our colleague Miss Sparrow (1952) has found that guinea pigs are extremely

insensitive to cortisone and that very large doses are needed to demonstrate even a moderate effect on the homograft reaction Papers which report failure to maintain homografts often note too, that granulation tissue, wound healing and the behaviour of autografts were normal Such remarks would seem to provide further evidence that the dosage of cortisone was inadequate The failure to obtain positive results with ACTH serves to underline the view that cortisone and ACTH should not be re garded as two interchangeable substances with equivalent effects

This paper has discussed only the particular problem of grafting homologous skin, where the difficulties in the way of successful grafts seem to be primarily due to im munological factors There are however, two other fields of interest that should be mentioned The first relates to the conditions that determine the survival of trans planted endocrine tissues Successful grafting of endocrine glands depends to a large extent on factors which are summed up in 'Halsted s law and which are concerned especially with the hormonal environment and only secondarily with immunological relationships The second relates to the study of immunity responses in pregnancy In a sense, the foetus *in utero* can be considered as a large homograft and it may be that changes in adrenocortical activity during pregnancy are related to the need for controlling the normal reactions of the body to such a homograft Both problems are likely to be elucidated best if the experimental approach is based on a combination of endocrinological and immunological methods

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Discussion

ON PAPERS BY (1) CAMERON, (2) KROHN

Chairman T F Hewer

T F Hewer We have heard a lot about the effect of ACTH in depleting adrenal ascorbic acid, can anyone tell me if there is any connection between this effect and the delay in wound healing seen in patients with ascorbic acid deficiency?

O A Trowell Professor Cameron has pointed out the fundamental importance of finding out the action of cortisone on different cell types

We have worked out a technique for studying the lethal effect of cortisone on lymphocytes *in vivo* We take 1–2 mm lymph nodes from young rats and culture them in a small perspex chamber in an atmosphere of oxygen, the nodes being supported on cotton wool moistened with serum saline These cultures will survive for four days without increase in the number of pyknotic cells above the baseline of 1–2 per cent We maintain them for two days and then transfer them to a medium containing cortisone, and examine the nodes five hours later In the first series of experiments we kept the time constant at five hours and plotted the cortisone concentration against the number of pyknotic cells

The minimum level of cortisone necessary to produce an effect was 0 1 microgram/ml, but increasing the cortisone concentration by a factor of 10 did not have very much more effect We then fixed the cortisone concentration at 1 microgram/ml and examined the nodes at different times We found that one gets a maximum effect after about forty six hrs in cortisone So we re tested at this time with varying cortisone dosages

If one now plots log dosage against pyknosis using a probit scale one gets a straight line Even at high cortisone concentrations only about 50 per cent of the cells are killed Compared with X radiation nitrogen mustard and cyanide the lethal effect of cortisone is a delayed one

By this method it is possible to detect 0 01 microgram of cortisone in 0 5 ml of medium

J M Yoffey The work of our own group seems to run counter to the general trend which has so far emerged in this session We started off by trying to repeat some experiments of Dougherty and White (1945 *Am J Anat* 77 81–116), on the effect of cortical extracts upon lymphocytes in blood and lymphoid tissue We were unable to repeat this work, and in fact with the cortical extract used we seemed to get an actual hypertrophy of lymphoid tissue (Yoffey and Baxter 1946 *J Anat* 80 132–8) Since that time Dougherty and White have withdrawn their original claims for a destructive effect of 11 oxycorticoids on lymphoid tissues Nevertheless some effect there is undoubtedly and we continued our studies directing attention to the level of the blood lymphocytes

There are two ways of trying to investigate the factors controlling the level of the blood lymphocytes One is to attempt to estimate the numbers entering the blood another to track down those which are leaving the blood Reinhardt and Li (1945—*Science* 101 360) adopted the first method, as we also did at first (Yoffey Reiss and Baxter, 1946 *Nature* 157 368) However we soon transferred attention to possible sites at which lymphocytes might be leaving the blood and since ACTH and 11 oxy steroids appeared to be having effects on several of the formed elements of the blood we turned to the bone marrow and attempted to devise a quantitative technique for the assessment of changes in it

The first experiments were performed with single injections of ACTH and cortical extracts It was found that there was no sign of diminution of lymphocytes in the bone marrow but on the contrary an increase (Yoffey Metcalf Herdan and Nairn 1951 *B M J* Vol 1 660) This result was unexpected and so we next tried repeated injections of ACTH giving injections once a day for seven days (Hudson, Herdan and Yoffey 1952 *B M J* Vol 1 p 999) There were no significant changes in marrow lymphocytes but the cellularity of the marrow as a whole was increased the change involving both the myeloid and the erythroid series but the latter more than the former In view of these findings it seemed advisable to perform a much larger number of experiments using individual steroid hormones Thirty guinea pigs were given 5 mg of cortisone daily for seven days ten were given a similar dose of Compound F and ten Compound A Again there was a tendency to increased cellularity of the marrow, involving both myeloid and erythroid cells but especially the erythroid The specific gravity of the marrow was significantly increased but there was no significant change in the marrow eosinophils or lymphocytes Compound A seemed to act as a more direct stimulus to granulocyte formation and granulocytes appeared to be forming directly from reticulum cells with a short circuiting of the typical myeloblast stage (Yoffey, Ancill Owen Smith and Herdan unpublished)

In short, ACTH and Compounds E F and A seem in the guinea pig—despite the statements usually made about the guinea pig being refractory to these substances—to have a stimulating effect on the marrow Whether, if the experiments were prolonged the erythropoietic stimulation would finally culminate in the development of a polycythaemia such as is not infrequently encountered in Cushing s syndrome we cannot yet say

K Aterman Professor Cameron has mainly dealt with the effect of cortisone on acute tissue changes Although the inhibitory effect of cortisone on inflammation is well known I feel that this cannot be the full answer I have therefore approached the question from another angle The continued administration of carbon tetrachloride to rats over a prolonged period of time leads to varying degrees of fibrosis of the liver I therefore gave carbon tetrachloride to two groups of rats over a period of about two months At the end of that time and while still receiving carbon tetrachloride one group was given cortisone for about ten days Despite the continued injection of carbon tetrachloride the degree of fibrosis in that group as seen on histological examination was appreciably smaller than in the group not receiving cortisone (K Aterman *Lancet*, 1951 p 517) I hope Professor Cavallero s group will bear me out on this point From this I can only deduce that although the chronic inflammatory changes present were also decreased a resorption of fibrous tissue must have

taken place Not only has no new fibrous tissue been formed, but there has been a decrease of the fibrous tissue that had already been deposited I have repeated these experiments in rats which were given carbon tetrachloride for about six months, but my results are only in the preliminary stage Of interest is that some of these rats died while receiving cortisone I am now trying to study the effect of cortisone on the fibrous tissue quantitatively to see if I can detect differences in the collagen content

G H Bourne I should like to give some information in answer to Professor Hewer s inquiry Connective tissue in an animal under treatment with cortisone does seem to undergo changes similar to that seen in animals with scurvy In the healing of a wound there is

1 A delay in the conversion of precollagen to collagen
2 A delay in the migration of phagocytes
3 A delay in the phagocytic process itself
4 A delay in the migration of fibroblasts

In scurvy there is a reduction of the tensile strength of healing wounds In rabbits with cortisone dosage similar to that given by Professor Cameron, we found that the tensile strength of a wound was reduced from 270–500 grms in the control, to 100–170 grms in the treated animals There was no overlap in the results, and we found that the administration of very large doses of ascorbic acid had no effect on these results This suggests that the mechanism whereby cortisone produces these connective tissue changes is different from that which produces them in vitamin C deficiency

I gather that Dr Smart has the impression that patients treated with cortisone even six months previously showed diminished tensile strength in wounds

G A Smart I would not base too much on that suggestion It was merely that a surgeon had told me about two recent cases in each of which the abdominal wound had burst open, and who had both, some time previously, been receiving cortisone or ACTH therapy I personally cannot accept this as evidence since it might well have been a coincidence

G L Montgomery I agree that the main effect of cortisone is on the cells and this is seen particularly in acute inflammation R Curran of my department has been working on the effect of cortisone on the tissue reaction to fibrogenic quartz dusts

He found that the injection of such a dust into the peritoneal cavity of a mouse gives an acute inflammation within twenty four hours and it is this acute inflammatory response which is inhibited by cortisone Phagocytosis also is depressed Mice given daily doses of 4 mgm cortisone still had the dust free in their peritoneal cavities three weeks later in a control animal most of the dust is phagocyted in a few days The onset of fibrosis was also delayed but once the fibrous tissue had formed, further cortisone failed to produce any effect on it

G Sala Dr Cavallero and I have confirmed in many tests the inhibitory effect of cortisone on mesenchymal cellular proliferation which has been stressed by Professor Cameron

In our experience cortisone inhibits also epithelial cell proliferation in fact we showed that in CCl_4 intoxicated rats, cortisone while reducing the fibrosis, as Dr Aterman reported inhibits also the proliferation of parenchymatous hepatic cells

Another remark I wish to make concerns the effect of cortisone on bone marrow— we showed that in rats cortisone (5 mg daily for twenty days) strikingly reduces the number of erythroid and myeloid cells The discrepancy between our results and those of Professor Yoffey could be due to the different doses used

K Aterman I can only repeat what I have said before I am not familiar with your experiments but from my findings I have drawn the conclusion that under certain circumstances at least fibrous tissue that has been deposited can be made to decrease under the influence of cortisone It should be pointed out that in my experiments I first induced the fibrous tissue formation and then gave fairly big doses of cortisone for about ten days

L R Broster It is always a source of worry to the surgeon in carrying out skin grafting to find enough skin My Registrar Mr T Hansen, is using amnion grafts in cases of varicose ulcers with excellent results I suggest greater use should be made of these grafts

G R Cameron I should like to congratulate Dr Trowell To have obtained results with such small amounts of cortisone is wonderful This sort of research is extremely valuable

We transplanted small amounts of spleen into the anterior chamber of the eye and found that in a few weeks a miniature spleen had formed there With by Trowell s standard relatively large doses of cortisone the miniature spleen turned yellow and had many pyknotic cells in about twenty four hours

I should like to ask Professor Yoffey what criteria he used for the identification of lymphocytes

Dr Aterman did you stop the CCl_4 injections whilst you were giving the cortisone?

K Aterman I did continue the carbon tetrachloride injections

G R Cameron Karunaratne and I showed that the fibrosis in rats livers produced by carbon tetrachloride will melt away on cessation of the injections provided that they were not continued for too long I believe six months was the limit

J M Yoffey In reply to Professor Cameron our criteria for the identification of lymphocytes were morphological We hope soon to publish our results in full together with photomicrographs depicting the different cell types

The Rôle of the Adrenal Cortex in Homeostasis

by

DWIGHT J INGLE

Research Laboratories The Upjohn Company Kalamazoo Michigan U S A

THIS paper is concerned with the following question What are the metabolic consequences of the increased secretory activity of the adrenal cortices when the organism is subjected to noxious stimuli (stress)?

The size and secretory activity of the adrenal cortex are controlled to a major extent by the amount of corticotropin released by the anterior pituitary The anterior pituitary adjusts its output of corticotropin to meet the physiological need of the organism for cortical hormones This need is minimal under non stress conditions but is increased by any type of stress During stress the anterior pituitary releases larger amounts of corticotropin and the secretory activity of the adrenal cortices is increased in proportion to the severity of the stress The mechanisms controlling the secretion and release of corticotropin are also sensitive to cortical hormone overdosage so that the release of corticotropin is inhibited and the adrenal cortices become inactive and atrophic This over simplified account of the interrelationship between the anterior pituitary and the adrenal cortex is shown in Figure 1 The mechanism of control of corticotropin secretion and release by the anterior pituitary is not known but it is probable that both neural and humoral components are involved [1]

SIGNS OF HYPERCORTICALISM

Experimental hypercorticalism can be induced by giving the organism an excess of either exogenous adrenal cortical hormones or of exogenous corticotropin During severe hypercorticalism the morphology and function of most if not all tissues of the body are affected Among the general changes there are inhibition of growth decrease in carbohydrate tolerance redistribution of fat and electrolyte and water imbalance Hypercorticalism was first produced by nature When malignant changes in the adrenal cortex cause it to secrete wildly Cushing s syndrome may ensue Most of the features of Cushing s syndrome have been found to occur in some patients with rheumatoid arthritis or related diseases who are given pharmacologic amounts of either cortisone or corticotropin over periods of weeks and months

Experimental hypercorticalism in the normal rat is illustrated by the glycosuria and the negative nitrogen balance induced by the administration of cortisone and of hydrocortisone (Figure 2)

Do the adrenal cortices of the rat have the capacity to secrete amounts of hormone which will cause comparable changes? Indeed so! Figure 3 illustrates glycosuria and a negative nitrogen balance in normal rats given corticotropin by continuous sub cutaneous injection [3] We have estimated [4] that the adrenal cortices of the rat can secrete the activity equivalent of at least 10 mg of cortisone acetate or 50 cc of beef adrenal extract per twenty four hours The adrenal cortices of either rats or man can secrete amounts of hormones sufficient eventually to cause death

CHANGES IN REQUIREMENT FOR CORTICAL HORMONES DURING STRESS

An amount of the cortical hormones which causes signs of hypercorticalism under resting conditions may represent adrenal cortical insufficiency during severe stress In our laboratories we have obtained indirect evidence that the adrenal cortices of the normal adult male rat secrete the activity equivalent of 3 to 5 cc daily of beef adrenal extract under non stress conditions Two criteria have been applied *A* When the mildly diabetic male rat on a constant food intake is adrenalectomized, there is a striking decrease in the level of urinary glucose From 3 to 4 cc daily of beef adrenal extract are required to restore the glycosuria to its preadrenalectomy level [5] *B* The presence of one intact adrenal inhibits the regeneration of a contralateral enucleated adrenal in an otherwise normal male rat In similar rats having one enucleated adrenal with the other adrenal removed an average of 5 cc daily of beef adrenal extract is required to inhibit regeneration of the enucleated adrenal as effectively as does the presence of one intact adrenal gland [6]

During severe stress the adrenalectomized rat requires much greater amounts of adrenal cortex extract or of steroids to sustain optimal resistance We[7] have studied the requirement for cortical extract in adrenalectomized rats subjected to faradic stimulation of the gastrocnemius muscle When the hormone was given by continuous intravenous injection, 20 cc of beef adrenal extract per twenty four hours were required to sustain a normal output of work Under similar conditions, 4 mg of cortisone or 2 mg of hydrocortisone per twenty four hours are required to support the maximal output of work that can be attained with these steroids

When amounts of exogenous cortical extract or of steroids such as are required to maintain optimal resistance to stress are administered to either intact or adrenal ectomized rats under non stres conditions, signs of hypercorticalism ensue The animal develops a negative nitrogen and potassium balance and loses weight there is extensive regression of the thymus and a lesser response in the other lymphoid organs, there are marked changes in certain blood elements the animal may develop a glycosuria its resistance to infection is lowered, and some animals develop gross pathology such as renal lesions and ulcers in the glandular portion of the stomach [8]

RÔLE OF THE ADRENAL CORTEX IN SOME OF THE METABOLIC CHANGES DURING STRESS

It is apparent that during severe stress the adrenal cortices secrete amounts of hor mones which are capable of causing metabolic upsets when given during non stress conditions Does the increased outpouring of these hormones only suffice to meet an

increased need during stress or are some of the metabolic effects of stress caused by the increased amount of the cortical hormones? We have carried out several experiments in our laboratories which relate to this question and provide a basis for a tentative answer

A Large doses of oestrogen are noxious to the rat and cause stimulation of the adrenal cortex via the discharge of corticotropin by the anterior pituitary Oestrogens cause exacerbation of glycosuria in the partially depancreatized force fed rat This response does not occur in the adrenalectomized depancreatized rat Activation of the adrenal cortex by the administration of exogenous corticotropin adrenal cortex extract or 11 oxygenated steroids causes exacerbation of glycosuria in the partially depancreatized rat On the basis of these observations it was postulated that activation of the adrenal cortex mediates the effect of oestrogen upon glycosuria The following experiment[9] was carried out Partially depancreatized rats which were without spontaneous glycosuria, developed glycosuria during the injection of 0 1 mg daily of diethylstilboestrol and the glycosuria disappeared when the injections were stopped The animals were then adrenalectomized and treated with a subdiabetogenic amount (3 cc daily) of adrenal cortex extract Glycosuria developed when diethylstilboestrol was injected and disappeared when the injections were stopped When the animals were maintained by treatment with either 11 desoxycorticosterone acetate or by drinking 0 9 per cent sodium chloride, the diabetogenic effect of the oestrogen was either slight or absent The data are shown in Figure 4 The study was repeated[10] on adrenalectomized hypophysectomized, partially depancreatized rats maintained on subdiabetogenic amounts of adrenal cortical and anterior pituitary extracts The administration of diethylstilboestrol caused hyperglycaemia and glycosuria in all of the test animals which were without glycosuria during the control periods, and in animals having spontaneous glycosuria it became more severe when the oestrogen was administered These data show that the oestrogen has a diabetogenic effect which is not mediated by a change in the secretory activity of the adrenal cortex but that the presence of cortical hormones is essential for the support of this metabolic response

B A similar problem has to do with the effect of oestrogen upon the growth of hair The systemic administration of large doses of oestrogen over a period of several weeks suppresses the growth of hair in rats Hair growth can be similarly inhibited by large doses of adrenal cortex extracts 11 oxygenated steroids or of corticotropin It is known that large doses of oestrogen cause stimulation of the adrenal cortex via corticotropin Hair growth is accelerated by adrenalectomy in the rat and is not inhibited by the administration of oestrogen in the adrenally insufficient rat A test was made of the hypothesis that the hair growth inhibiting effect of the oestrogen was mediated by an increase in the secretory activity of the adrenal cortices Here again it was shown[11] that the cortical hormones play a supporting role for when the adrenal glands of rats were removed and the animals treated with adrenal cortex extract the hair growth inhibiting effect of the oestrogen was sustained The results are illustrated in Figure 5

C Among the metabolic responses to injury there is a rise in the excretion of nitrogen and a temporary inhibition of the excretion of sodium and chloride Since the adrenal cortical hormones can stimulate nitrogen loss and cause sodium and

chloride retention, we tested the hypothesis that these responses were due specifically to an increased outpouring of adrenal cortical hormones during the stress In one experiment[12] it was shown that following fractures the characteristic rise in urinary nitrogen fails to occur in adrenally insufficient animals, but it does occur full blown in similar animals maintained on adrenal cortex extracts In a subsequent study[13], it was shown that the adrenalectomized rat treated with a uniform intake of adrenal cortex extract throughout the experiment responds normally to fractures by an immediate, but temporary, retention of sodium and chloride and a rise in urinary nitrogen These results are summarized in Figure 6

D The 11 oxygenated steroids of the adrenal cortex are diabetogenic It has been postulated that the increased secretory activity of the adrenal cortices during stress should cause exacerbation of diabetes The hypothesis was tested by comparing the responses of adrenalectomized and nonadrenalectomized rats to the administration of solutions of formaldehyde[14] Male rats were made mildly diabetic by partial pancreatectomy and were force fed a medium carbohydrate diet Fifteen rats were adrenalectomized and treated with adrenal cortex extracts in amounts which sustained the preadrenalectomy level of glycosuria An equal number of nonadrenalectomized rats were studied in parallel The subcutaneous injection of solutions of 1 5 per cent formaldehyde, in doses of 0 25, 0 5 and 1 cc twice daily for seven days five rats per dose level, caused some decrease in the glycosuria of the nonadrenalectomized rats and a much more striking decrease in the glycosuria of the adrenalectomized rats When the injections were stopped, the glycosuria was re established at its preinjection level The data are shown in Figure 7 It seems probable that the increased output of cortical hormones during the stress did have a positive effect upon the level of urinary glucose in that it tended to prevent the marked decrease in glycosuria which occurred in the adrenalectomized rats These adrenalectomized rats which were on a fixed intake of cortical extract—an amount which represented full replacement under non stress conditions—became adrenally insufficient during the increased need for more hormone, and the glycosuria fell sharply as is characteristic of adrenal cortical insufficiency These data add further support to the concept that the increased secretory activity of the adrenal cortices during stress tends to maintain homeostasis rather than to cause hypercorticalism

COMMENT

According to our tentative conclusion the excursions in adrenal cortical activity which occur in response to various noxious stimuli serve to meet changing needs of the organism for these hormones and do not cause hypercorticalism The metabolic responses to stress are not caused primarily by an increased outpouring of the cortical hormones although the presence of the cortical hormones may be necessary for the response to occur

This concept that corticotropin and the hormones of its target organ the adrenal cortex play a supporting role rather than a prepotent regulatory role in metabolic adjustments has been supported by a number of other observations which have been reviewed and extended by Engel[15] and by Sayers[16]

There may be exceptions to the above generalizations It is probable that the

regression of lymphoid tissue, and the fall in blood eosinophils and lymphocyte which occurs during stress is due to activation of the adrenal cortices However, these signs of adrenal cortex response to stress are not effected exclusively by the cortical hormones

Selye[17] has postulated that the adrenal cortex response to stress may be an important etiologic factor in many diseases This concept will not be discussed in detail here There is no doubt that the presence of the adrenal cortical hormones is essential for the overt manifestation of such diseases as diabetes mellitus hypertension prostatic cancer, etc It may be possible to explain much of the evidence which Selye *offers in support of his hypothesis in terms of the permissibility of cortical hormone* action In any case there are major gaps in the evidence for the interesting concept that derailment of adrenal cortex function is an important cause of frequently occurring diseases

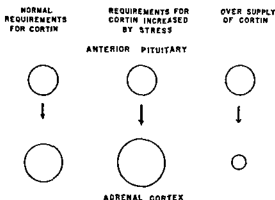


Figure 1 Diagram of the general functional interrelationship between the anterior pituitary and the adrenal cortex

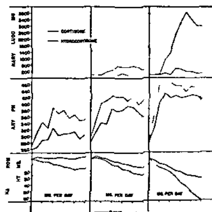


Figure 2 Data on adrenal steroids given by continuous subcutaneous injection to normal force fed male rats Average for six rats per group

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This concept that corticotropin and the hormones of its target organ, the adrenal cortex, play a supporting role rather than a prepotent regulatory role in metabolic adjustments has been supported by a number of other observations which have been reviewed and extended by Engel[15] and by Sayers[16]

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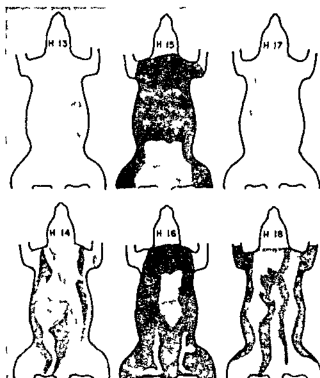


Figure 5 Drawings of hair pattern grown during the fourth week following operation H 13 sham adrenalectomy oestrogen H 14 sham adrenalectomy control H 15 adrenally insufficient oestrogen H 16 adrenally insufficient control H 17 ACE treated adrenalectomized oestrogen H 18 ACE treated adrenalectomized control

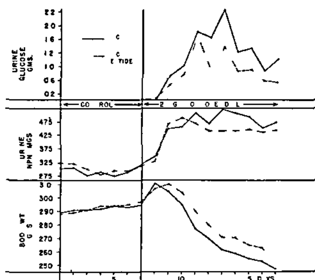


Figure 3 Comparison of the effects of two different forms of corticotropin given by continuous subcutaneous injection for ten days Averages for six rats per group [3]

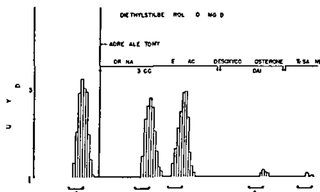


Figure 4 The diabetogenic effect of diethyl stilboestrol before and after adrenalectomy of the partially depancreatized rat as influenced by the nature of the replacement therapy Average value for five rats [9]

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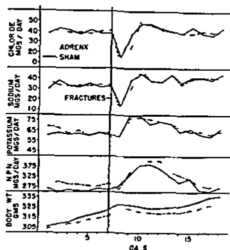


Figure 6 Metabolic responses to fractures in non adrenalectomized and adrenalectomized force fed rats The adrenalectomized rats received 4 cc of ACE per rat per day throughout the experiment Averages for six rats per group [13]

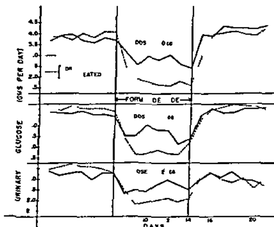


Figure 7 Effects of a stress (injections of 1 5 per cent formaldehyde) on glycosuria of depancreatized rats and depancreatized adrenalectomized rats treated with ACE Each average is based upon five rats [1]

The Influence of the Suprarenal Cortex on Mineral and Water Metabolism

by

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THE effects of adrenalectomy and the administration of cortical steroids on mineral balance have already been mentioned by previous speakers But little has been said about the mechanisms underlying these effects The kidney is the main organ con cerned with the regulation of the electrolyte and water metabolism and attention will therefore naturally be first focused on renal function

Fig 1 shows the effects of adrenalectomy on glomerular filtration rate (= GFR) renal blood flow (= RBF) tubular secretion (as measured by clearance techniques) and water diuresis It will be seen that all these functions are considerably depressed in the adrenalectomized dog and similar results have been reported in rats (Fried man, MacKenzie and Friedman 1948 Boss Birnie and Gaunt 1949) The relation ship between changes in systemic blood pressure and the state of the renal vessels and the decrease of GFR needs further exploration Nor is it possible to decide to what degree the decreased tubular secretory function is due to circulatory changes Tm_D or Tm_{PAH} (= maximal rate of tubular excretion of diodone or p aminohippuric acid) in the adrenalectomized or adenohypophysectomized dog is low enough to make one suspect that factors other than vascular will have to be adduced but this is doubtful in other species e g in man Fig 1 shows further that qualitatively adeno hypophysectomy has the same effects on the renal function under review as removal of the adrenal cortices Fig 2 summarizes data on renal function and water diuresis in patients suffering from Addison s disease and hypopituitarism It will be seen that the changes produced by these diseases are very similar to those observed in adrenal ectomized and hypophysectomized animals It should be noted that the data given in this and the preceding figure are for various reasons to be regarded as indicating no more than the order of magnitude of the changes observed by several groups of investigators Even so the similarity between the results in patients and experimental animals suggests that it is a fair procedure to use the latter for evaluation of prepara tion intended for substitution therapy in the diseases mentioned A survey of the literature leaves one with the impression that such work has been done to a lesser extent than seems desirable In fact it would seem at present that more comprehen sive data on the effects of adrenal preparation in cortical deficiency are available from clinical trials Their interpretation is not only complicated because doses administered and duration of treatment varied from investigation to investigation but also because the degree of deficiency varied from patient to patient

fluids and perhaps chiefly of the extracellular fluid (Smith 1951) have been postulated and it is conceivable and even likely that changes in renal sodium and potassium excretion are involved in this process However the evidence at present available is insufficient to assess this possibility and until it comes forth one will I feel, do well to remember how difficult it is to gauge primary extrarenal changes in the presence of alterations of renal function

Since it has been shown that injections of cortical extract control the effects of adrenalectomy and Addison s disease on the electrolyte metabolism the question arises to which steroid or steroids this action should be attributed Lack of time does not permit the discussion of this problem Suffice it to say that one group of very recent investigators (Tait Simpson and Grundy, 1952) comes to the conclusion that the activity of adrenal cortical extracts on electrolyte metabolism is due to some factor other than the known crystalline cortical steroids while others (Roberts and Pitts 1952) have reported that electrolyte balance in adrenalectomized dogs together with other impairments of renal function can be restored by cortisone provided only that sufficiently large doses of this steroid are given for a sufficiently long period

In conclusion some remarks on a problem which has raised much controversy in recent years I mean the question whether the decreased water diuresis due to increased tubular water reabsorption (Wirz 1945 Roemmelt Sartorius and Pitts 1949 Lotspeich 1949, Boss Birnie and Gaunt 1950) in adrenal cortical deficiency can be related to the posterior pituitary gland Conceptually several mechanisms or their combinations may be invoked to account for such a change in endocrine balance The disturbed water metabolism in adrenal insufficiency may lead to increased release of the antidiuretic hormone In favour of this possibility are the results of Gaunt and his co workers who found a higher antidiuretic potency in the heart blood of adrenalectomized rats than in intact controls However Fig 4 shows that ether anaesthesia which was used by these workers may apparently act as a secretory stimulus (Ginsburg and Heller 1952) Moreover we have evidence that the removal of large quantities of blood by heart puncture also favours the release of the antidiuretic principle It may thus be argued that the results of the American investigators are due to an increased response of the neurohypophysis to noxious stimuli after adrenalectomy The matter requires reinvestigation with more sensitive methods of assay and in other species It cannot be solved by determining the antidiuretic potency of peripheral blood since it cannot be excluded that adrenal deficiency lowers the rate of inactivation of the antidiuretic hormone This possibility has been postulated by Birnie (1950) who found that the liver of adrenalectomized rats is less potent than that of normal animals in inactivating vasopressin Fig 5 shows that *in vivo* not only the liver but also the kidney participates in the removal of injected vasopressin and results of my colleague Dr Ginsburg (Fig 6) indicate that the removal of injected vasopressin is slower in adrenalectomized animals These experiments are open to the objection that the doses of vasopressin injected (100 milliunits per 100 g rat) were unphysiologically high and that the endogenous hormone may behave differently But they would be in accord with those workers (Corey Silvette and Britton 1939 Birnie Eversole Bos Osborne and Gaunt 1950 Lockett 1952) who found that the sensitivity of adrenalectomized animals to small doses of vasopressin is increased Chalmers and Lewis (1951) on the other hand

Fig 3 shows that treatment of Addisonian patients with salt, deoxycorticosterone or commercial adrenal extracts does not have a consistent effect on such renal functions as RBF, GFR, tubular secretion or tubular water reabsorption The amounts of deoxycorticosterone or adrenal extract may have been insufficient or the duration of administration too short, but against this is the fact that disturbances of the sodium and potassium metabolism were controlled with these doses and that the clinical state of the patients had much improved Another possibility has been considered, viz, that the renal impairment had become irreversible by the time therapy had been started That seems unlikely in view of recent reports that ACTH given to patients with hypopituitarism raises GFR and RBF significantly (Hellman Weston, Escher and Leiter 1948, Levitt and Bader, 1951) and brings water diuresis almost to normal (Chalmers and Lewis, 1951, Oleesky and Stanbury, 1951), whereas administration of deoxycorticosterone to such cases does neither In Addison's disease cortisone has been shown to increase water diuresis very substantially (Chalmers and Lewis, 1951) and Roberts and Pitts have reported quite recently (1952) that prolonged administration of large doses of cortisone to adrenalectomized dogs restores GFR and elevates RBF Moreover an increase of GFR and RBF and water diuresis after cortisone has been reported (Ingbar *et al*, 1950, Levitt and Bader 1951) to occur in subjects not suffering from adrenal or pituitary impairment

It is generally accepted that the renal excretion of electrolytes and in particular that of sodium and potassium is disturbed in adrenal deficiency sodium excretion by the kidney is increased and potassium excretion is diminished

How are these alterations in renal function brought about? Are they due to the *circulatory changes* already discussed? These may be a factor in gross adrenal deficiency but changes in the rate of sodium and potassium excretion persist in well stabilized animals with GFR values as high as in intact controls The *plasma concentrations* of sodium and potassium cannot be regarded as decisive since excessive sodium excretion continues at subnormal plasma levels and retention of potassium at increased ones Does the lack of cortical secretion lead to *interference with the unknown metabolic processes underlying active ion transfer* in the renal tubules? This hypothesis is difficult to reconcile with the results of Roemmelt Sartorius and Pitts (1949) who have shown that the adrenalectomized dog when given salt is able to reabsorb a greater percentage of sodium than the intact animal The evidence on potassium is less clear Tubular secretory processes may be involved in potassium excretion at high plasma levels and it has already been pointed out that the secretory tubular transfer mechanisms are impaired in adrenal deficiency It remains to consider whether the changes in the renal excretion of the two cations are the result of the *distortion of the extracellular fluid space and the extrarenal electrolyte distribution* Such changes have been repeatedly demonstrated in adrenal insufficiency (Swingle Parkins Taylor and Hays 1936, Harrison and Darrow, 1938 Flanagan and Overman 1949 Gaudino and Levitt 1949) and it has been concluded on the basis of these findings and as a result of observations on the effect of adrenal cortical extracts and corticosteroids that the adrenal cortex exerts its primary influence on cell permeability (for reference see Davis Bass and Overman, 1951 Levitt and Bader 1951 Overman Davis and Bass 1951) and hence on the equilibrium distribution of fluid between the extracellular and intracellular phases Renal mechanisms for the regulation of the volume of body

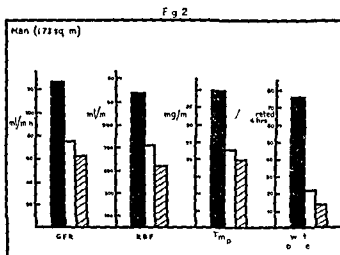


Figure 2 Glomerular filtration rate (= GFR) effective renal blood flow (= RBF) maximal tubular transfer of diodone (= Tm_D) and water diuresis in patients suffering from Addison's disease or hypopituitarism The columns represent means calculated from the data of Talbott, Pecora Melville and Consolazio (1942) Sanderson (1948) Waterhouse and Keutman (1948) Slessor (1951) and Smith (1951)

■ = normal □ = Addison's disease

▨ = hypopituitarism

failed to find an increased antidiuretic response to vasopressin in patients suffering from Addison's disease or hypopituitarism, but it must be considered that comparisons of antidiuretic effects produced by vasopressin are very difficult when the rate of water and electrolyte excretion differ markedly However, even assuming that an increased response to the antidiuretic principle can be established in adrenal cortical deficiency it may still be unrelated to the rate of inactivation of the neurohypophysial hormone but may be due to a change in the responsiveness of the tubular effector cells to the hormone Finally, the increase of tubular water reabsorption in adrenal deficiency may not be linked with the neurohypophysis at all but may be the result of some undisclosed changes in the kidney The problem is thus an open one It has recently been further complicated by (unpublished) results of my Italian co workers, Drs C Cavallero, E Dova and L Rossi, who found a significant depletion of the neurohypophysis in adrenalectomized rats However, it remains to be seen whether their findings mean more than that the neurohypophysis after the release of hormone due to the stimuli of anaesthesia (see Fig 4) and surgical measures, is more slowly repleted in the adrenalectomized animals than in sham operated controls

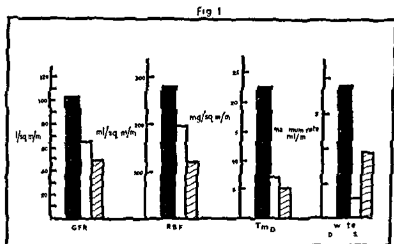


Figure 1 The effects of adrenalectomy or hypophysectomy in dogs on glomerular filtration rate (= GFR) effective renal blood flow (= RBF), maximal tubular transfer of diodone (= Tm_D) and water diuresis Data from papers of White Heinbecker and Rolf (1942 1947) Heinbecker, White and Rolf (1943) and Pickford and Ritchie (1945) were used

■ = *normal* □ = *adrenalectomized*

▨ = *hypophysectomized*

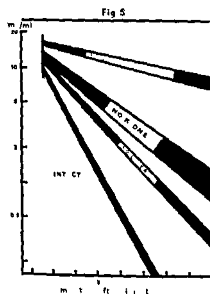


Figure 5 The clearance of intra enously inj cted vasopressin from the circulating blood Anaesthetized rats were injected intra enously with 100 milliunits of vasopressin per 100 g body weight and small volumes of blood were withdrawn from a cannulated carotid artery at short intervals after the injection The antidiuretic activity of these samples (and that of control samples taken before the injection) were estimated by immediate intra venous i jection into unanaesthetized rats with high water loads Results are shown for intact animals animals in which the li er was excluded by ligation of the coeliac superior mesenteric and inferior mesenteric arteries rats in wh h both kidneys had been removed and rats in which both the liver and the kidneys had been excluded from the circula ion The results shown are of 4 – 5 experiments ± their standard error The ordinate shows on a lo arith r scale concentration of antidiuretic activity in terms of milliunits vasopressin per ml blood It will be seen that in the intact animals the antidiuretic activity ceased to be traceable about five minutes after injection The clearance of the injected vasopressin was markedly delayed when the li er was excluded from the circulation and even more so when the kidneys had been eliminated (Ginsburg and Heller unpublished)

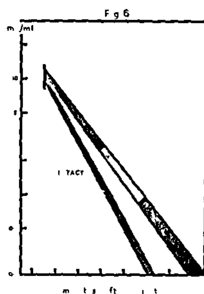


Figure 6 The experiments were done in the same manner as those described in Figure 5 Means with their standard error are shown The dose of vasopressin gi en was again 100 milliunits per 100 g rat Bilateral adrenalectomy (= ADX) had been performed forty ei ht hours before the injection of vasopressin It will be noted that in the adrenalectomized rats injected vasopressin was cleared from the blood at a significantly lower rate than in the controls (Ginsburg unpublished)

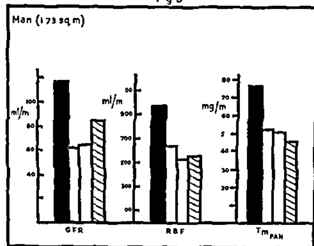


Figure 3 Effect of therapy in Addison s disease on glomerular filtration rate effective renal blood flow and maximal tubular transfer of p aminohippuric acid (= Tm_{PAH}) *Data of Waterhouse and Keutman* (1948)

= *healthy controls*

= *Addison s disease with salt therapy*

= *Addison s disease and DCA*

= *Addison s disease with adrenal cortical extract (aqueous)*

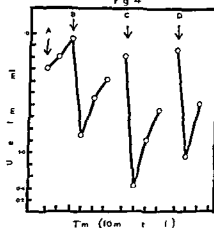


Figure 4 Comparison of the antidiuretic effect of blood obtained from an unanaesthetized rat and after the same animal had been anaesthetized with ether The blood samples were withdrawn from a polythene cannula in the donor s right external jugular vein and were immediately injected into a recipient with a similar cannula The recipient was kept at a high rate of water diuresis by repeated doses of water by mouth

A = 0 3 *ml external jugular blood from unanaesthetized rat*
B = 0 3 *ml external jugular blood from same donor in ether anaesthesia*
C = 0 2 *milliunits vasopressin (per animal)*
D = 0 1 *milliunits vasopressin (per animal)*
(Ginsburg and Heller unpublished)

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Discussion

ON PAPERS BY (1) INGLE (2) HELLER

Chairman G F Marrian

G F Marrian I should like to ask Dr Ingle whether he used any natural oestrogens in his experiments

D J Ingle Yes we tried oestradiol oestrone and synthetic oestrogens of different chemical structure and we found that anything oestrogenic had the same effect on the carbohydrate metabolism

Dr Aterman Dr Ingle s interesting paper prompts me to report an unusual manifestation of hypercorticalism in the rat which may have some bearing on a disease sign in

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mgm per day the tolerance test improved and after three months treatment with 50 mgm per day, a nearly normal curve was obtained

The last case is another example of diabetes in a bearded woman The diabetes was slight, no insulin was given Under a first cortisone treatment the blood sugar decreased The patient was then put on 6 25 mgm cortisone without effect Treatment with 25 gm, brought again a reduction of the fasting blood glucose and an improvement in the glucose tolerance test But increase to 50 mgm was followed by a definite aggravation of the diabetic condition

We think that these cases may be examples of steroid diabetes and that the exogenous cortisone administration temporarily inhibited the endogenous output of sugar hormones

G A Smart The mineral regulating effect of the suprarenal cortical hormones has been related by Professor Verzar to a primary effect on carbohydrate metabolism He thought that the stimulation of cellular carbohydrate metabolism resulted in a transfer of the potassium to an intercellular position and suggested that the electrolyte effect was entirely secondary to this

I would like to ask Professor Verzar if he could explain the following clinical findings It is common to find cases of Addison s disease who are adequately controlled as far as their mineral balance goes, but who have spontaneous hypoglycaemia One such patient whom I saw recently was over controlled as shown by sodium retention and oedema but still had hypoglycaemia

We have been investigating the total body potassium of normals of patients with Addison s disease and of patients with Cushing s disease using the radioactive potassium technique, in which one injects a known amount of radioactive potassium waits for equilibrium to take place and then estimates the total body potassium from the equilibrium condition between radioactive and natural potassium This procedure also gives one some idea of rate of diffusion of potassium as measured by the time for equilibrium to take place, between the injected and tissue potassium

We found the normal level of potassium to be 45–46 milliequivs per kilogramme of body weight which agrees quite well with Corsa *et al* at Boston who found levels of 37–51. We have had two cases of untreated Addison s disease in which the levels were 56 and 57 milliequivs respectively if Professor Verzar s theory were correct surely intracellular potassium levels should be lower than normal? We had two cases of Cushing s disease who had body levels of 29 and 31 milliequivalents of potassium respectively It is true that people with Cushing s disease are fat and we do not know the potassium level of fat but this finding would agree with the fact that the muscle potassium is low in Cushing s disease

The time taken to reach equilibrium, which may be some measure of cell membrane permeability is about eighteen hours in normals and in Addison s disease but seems to be a good deal more than twenty four hours in Cushing s disease

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the human I refer to experimental exophthalmos which has only rarely been reported in the rat

The work reported here was undertaken in association with Dr S M Greenberg of the University of Southern California I first observed marked bilateral exophthalmos about a year ago *in one of my rats (with carbon tetrachloride fibrosis of the liver)* which had been treated for five days with cortisone On the sixth day it showed definite proptosis confirmed by three observers On the eighth day, however, the proptosis had disappeared Since in the rat exophthalmos cannot be measured I decided to investigate this problem in the guinea pig which is a more suitable animal

When Dr Greenberg and I undertook a study on the effect of the prolonged administration of cortisone on the weanling male rat, we again noticed that a good proportion of our animals receiving 1 5 mg of cortisone daily for six days a week for four weeks showed what we took to be proptosis At first we had to rely entirely on photographic records but with increasing experience we found it possible to assess the exophthalmos fairly accurately without photographs Three of us, Dr Greenberg, myself and my assistant were handed animals of which we knew only the numbers We assessed the proptosis independently and then compared notes I should like to add that the proptosis was bilateral, frequently persisting but occasionally disappearing again

One animal proved to be of great interest It had developed bilateral exophthalmos rather early but on the thirteenth day of its treatment with cortisone it showed unmistakable unilateral proptosis, which must have occurred quite rapidly At first we thought that the proptosis was caused by a tumour or by some infection but on histological examination of the retro orbital tissues we could find no evidence of either, and we therefore conclude although rather hesitantly, that this unilateral proptosis may be analogous to the well known but rare unilateral endocrine exophthalmos

P A Bastenie I would like to give a short report of three cases of diabetes in which cortisone treatment had a paradoxical effect

The first patient was a seventy year old woman with an Achard Thiers syndrome (diabetes in bearded women) Her diabetes was slight and was easily controlled with twelve unit insulin She suddenly developed subacute rheumatic arthritis with fever and increased sedimentation rate Cortisone was given in doses of 100 mgm per day In view of the unanimous reports on aggravation of the diabetic condition in the few cases which have been so treated, the blood sugar was checked daily To our great surprise it decreased together with the glycosuria

After fifteen days treatment without increasing the insulin doses the fasting glycosuria had dropped from 170 to 130 mgm per cent The day after treatment was suspended, it went down to 110 mgm After ten days rest treatment with ACTH was given, 50 mgm per day for ten days The blood sugar went up at once acetone appeared in the urine and the doses of insulin had to be doubled After a new control period, a second course of cortisone treatment was given After a short initial rise the blood sugar decreased again, reaching 140 mgm on the tenth day of treatment and falling to 90 mgm on the fifteenth day after stopping the treatment

The second case was a patient with rheumatoid arthritis She was obese had hypertension but no beard There was no actual diabetes but the glucose tolerance curve before treatment had a diabetic like shape Under treatment with cortisone 100

The flexibility of the system is not restored by DCA which only corrects the impaired sodium reabsorption of adrenal deficiency without restoring the diuretic response or the power of the kidney to concentrate These functions are restored by cortisone and by the adrenal steroids formed under the stimulus of ACTH

D J Ingle I am afraid I cannot add much clarity to the proceedings I do not think these suprarenal hormones have an obligatory effect on metabolism Under certain conditions the response will go in one direction and under other conditions the direction of the response may be reversed

Years ago I carried out a series of experiments in which moderate doses of adrenal extract administered to partially depancreatized rats caused a small temporary decrease in the glycosuria of some of the rats We interpreted this as due to a decrease in adrenal secretion of glucocorticoids In another study I found that the administration of large amounts of DCA to diabetic animals exacerbated the diabetes whilst smaller doses decreased it

I agree that these hormones affect both the mineral and carbohydrate metabolism but at different dosage levels and to different extents It is impossible to dissociate mineral from carbohydrate metabolism in different experiments because of the basic interrelationships between organic and inorganic metabolism

H Heller I welcome the very interesting concept of Dr Lewis though it is partly, as he emphasized himself founded on Homer Smith s interpretation of the function of the renal tubular segments, with which not all of us are in full agreement

F Verzar I am not at all sure that work on patients with Addison s disease can be directly compared with work done on adrenalectomized rats A physiologist tries to work on a relatively simple system The facts on which I based my lecture yesterday were that in isolated organs, e g yeast cells, and leucocytes there is a parallelism between the carbohydrate and mineral metabolism Dr Ingle's experiments were rather similar to ours Nerve and muscle physiologists find a similar parallelism All the isolated suprarenal hormones have some effect on both the carbohydrate meta bolism and the mineral balance

Is it not possible that the discrepancies are due to the fact that the carbohydrate and mineral activities may not have the same time relationships?

H Heller I do not think one should really rely too much on the plasma levels of potassium, as potassium readily moves from one compartment of the body to another The absolute amount in the body may depend on the relative sizes of the various body compartments

A A G Lewis In adrenal deficiency, there is a failure of the normal diuretic response to ingested water This also occurs in hypopituitarism, and Dr Chalmers and I [CHALMERS, T M and LEWIS, A A G (1951) The effect of adrenocorticotropic hormone on the diuretic response to water in panhypopituitarism *Lancet* 2 1158–60] have shown that ACTH will restore the response in this condition But this failure of the kidney to secrete a large volume of dilute urine is not the sole defect these patients cannot pass a very concentrated urine when deprived of water We have evidence that ACTH and cortisone will restore this capacity also

There is, therefore a lack of flexibility in the renal response to demands for water excretion or conservation It is this that has led us to reject the theory that increased activity of the anti diuretic hormone is the only cause for the absent diuretic response in adrenal deficiency

In the normal subject, 18 mEq of sodium in 120 ml of water are filtered at the glomerulus every minute If we take Homer Smith s views of tubular function as a working hypothesis (and they are supported by a good deal of evidence) about 15 mEq of this sodium are reabsorbed in the proximal tubule, apparently indepen dently of hormonal influence 100 ml of water are here reabsorbed secondarily, as the result of osmotic forces The distal tubule therefore receives about 3 mEq of sodium a minute, in 20 ml of water Here, further sodium reabsorption takes place under the influence of cortical hormones, but water reabsorption no longer occurs solely as a consequence of this—it is controlled by a separate hormone, ADH This separation of distal tubular water and sodium reabsorption and the control of each by a distinct hormone, means that they can be varied independently of one another thus during a water diuresis the volume of urine formed per minute rises and falls while the sodium excretion remains unaltered or falls slightly Flexibility is thus im parted to the system

Our suggestion is that 11 oxysteroids are necessary for this differentiation of distal tubular function—in their absence, the sodium reabsorption leads to water reabsorp tion, and possibly vice versa, the function of the distal tubule coming to resemble that of the proximal much more closely in this respect The volume of urine is there fore reduced and much less variation in its sodium concentration is possible

Surgery of the Adrenal Gland

by

L R BROSTER

Charing Cross Hospital, London

THE surgery of the adrenal like that of the other endocrine glands is mainly confined to the overactive states If by clinical correlation and observations on the reaction to treatment it has opened up a wider field for specialized study then we may regard it as having served a useful and introductory purpose

HISTORICAL

Changes in sex have been recorded from time immemorial and no review of this subject and its ramifications is complete without some reference to its historical background Cawadias (1943) states that according to Hippocrates the ancient Eastern peoples regarded them with superstitious awe and reverence and as examples of divine will and intervention

The Greeks were more rational and discarded these supernatural ideas although in Sparta monstrous children were put to death From Roman times to the Renaissance these individuals were regarded as manifestations of divine anger and the harbingers of national calamity and in order to propitiate the gods they were put to death Indeed, in 1803 when the King Henry VII chapel at Westminster Abbey was being restored, among some hundred figures that of St Wilgefort was found intact She in order to escape an unwelcome marriage prayed that she might become ugly and grew the beard with which she is shown Her father the King of Portugal was so irate that he had her crucified!

Literature art and sculpture are full of such representations real fantastic and mythological The reaction of modern society although more tolerant and enlightened is still one of reserved aloofness Some of the perversions are denied equal legal status and there is no organized scheme for dealing with them

From long observation based on our own out patient department it would seem that many of these patients are the lineal descendants of Pandora carrying the genes real or potential of all the human distempers contained in her box There is no human spectacle more distressing or pathetic than the individual otherwise healthy who is condemned through no fault of his own to live the life of a social pariah or is chained to an environment in which he is a complete misfit On the other hand there are some who contend with their adversity, with a courage and steadfastness that would rank high among the human virtues of Prometheus The imitators rather than the genuine cases are those which merit our contempt

In medical science sex changes have long been observed in association with adreno cortical tumours In this country Bullock and Sequeira (1905) added important

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Literature art and sculpture are full of such representations real fantastic and mythological The reaction of modern society although more tolerant and enlightened is still one of reserved aloofness Some of the perversions are denied equal legal status and there is no organized scheme for dealing with them

From long observation based on our own out patient department it would seem that many of these patients are the lineal descendants of Pandora carrying the genes real or potential of all the human distempers contained in her box There is no human spectacle more distressing or pathetic than the individual otherwise healthy who is condemned through no fault of his own to live the life of a social pariah or is chained to an environment in which he is a complete misfit On the other hand there are some who contend with their adversity, with a courage and steadfastness that would rank high among the human virtues of Prometheus The imitators rather than the genuine cases are those which merit our contempt

In medical science sex changes have long been observed in association with adreno cortical tumours In this country Bullock and Sequeira (1905) added important

contributions to the pathology, and Holmes (1925) published the case of the first successful removal of adenoma However, the important problem was to find the reason why some of these tumours produced sex changes, and others did not It was from this point that our inquiry started

CLINICAL

The adrenal cortex produces sex changes in three different directions, and the degree of change depends on the time of onset, and on whether the lesion is a hyperplasia or a tumour Clinically the most common condition is the masculinization of the female Feminization of the male is very rare, sexual precocity in children is uncommon Although the adrenal syndrome may remain predominant it is essentially polyglandular in nature for sooner or later secondary manifestations may arise from the other glands

THE ADRENOGENITAL SYNDROME

In this syndrome two changes take place concurrently female development and function are suppressed while the secondary sex characters of the male become superimposed Here we recognize four different groups according to the time of onset (Broster *et al*, 1932)

I When the changes occur either before or at puberty the feminine characters and functions are completely suppressed, and the figure of the girl resembles that of a boy

II In the following groups a girl passes through puberty normally, then usually within the next two decades the changes commence insidiously, and are as a rule ushered in by the appearance of hair of the male type and an upset in the feminine function

III In the third or Cushing group the onset is similar, but instead of remaining muscular and virile, secondary changes of a metabolic type set in resulting in adiposity a high blood pressure a low sugar tolerance polycythaemia, cutaneous discoloration and striae (Broster, 1940)

IV To these may be added the menopausal group the bearded old ladies so commonly found in asylums

The more detailed signs and symptoms of the adrenogenital syndrome may be summarized as follows

a Male changes

Hair of the male type and distribution begins to grow and baldness may supervene later The texture of the subcutaneous tissue and skin coarsens and acne spots are common The bodily contour tends to become masculine, with broad shoulders and narrow hips In the sexual sphere the voice deepens and the clitoris is enlarged

b Female changes

There is a general retrogression in the feminine characters The breasts do not grow the external genitalia are poorly developed the uterus may be infantile the ovaries become cystic and degenerate The menses may never start, or they may become irregular scanty and cease Fertility is decreased

c Associated with these somatic changes are certain psychological reactions The majority have headaches and are depressed, retiring and self conscious Some suffer from a sense of inferiority and avoid the society of men Others develop an ascendency over women folk and become homosexual A few develop psychosis of the paranoid type The incidence of psychosis in these women has been about 17 per cent in contrast to the normal incidence of about 1 per cent

FEMINISM

We have observed a few cases of this type where there has been a retrogression of male characters after puberty The external genitalia are small the skin is smooth and hairless, the beard does not grow the voice does not crack the limbs are long and spindly there is tapering of the fingers and fat is deposited on the chest and buttocks (1941)

ISOSEXUAL PRECOCITY

In children isosexual precocity of adrenal origin is due to carcinoma It must be stressed that carcinoma may arise in a solitary gland These sexually mature children with their childish minds the so called pocket Herculeses and Venuses are pathetic products of humanity They are dusky complexioned with torsos out of proportion to their limbs They appear to compress a decade of development into each year of their life

Boys develop pubic and somatic hair at an early age the voice is deep the genitalia over developed and they are precocious in their overtures to the opposite sex Girls develop breasts pubic hair and menstruate early Their coy and coquettish be haviour before a class of students may produce consternation among adult females in a ward Isosexual precocity may be idiopathic, or due to hypothalamic tumour In girls thecal tumours of the ovary and in boys interstitial tumours of the testis may also be the causative factor

TUMOURS

In tumours of the adrenal cortex the sex changes are as dramatic in their appearance as in their disappearance after removal of the tumour

The most satisfactory are the rare and simple adenomas Carcinomas may not run true to clinical type In children apart from isosexual precocity there may be also heterosexual precocity especially when the opposite adrenal is present

In the adult carcinoma may give rise to Cushing s syndrome, marked hirsutism only in the female (Broster 1950) or feminism in the male (Simpson 1938) It is an interesting reflection that these tumours in their riotous change of structure should retain their physiological function which is also present in their secondary deposits

Surgical treatment by adrenalectomy was started at Charing Cross Hospital in 1926 and we soon became involved in matters of cause and effect In the first place we had to find what the cause was and in the second place to explain the amelioration in the condition after operation

PATHOLOGY

In 1931, Professor Vines took over the pathological study of the adrenal glands removed at operation, and found that by means of the Ponceau Fuchsin stain the hyperplastic cortical cells in virilism stained a vivid red colour in contrast to the blue stain present in normal controls (1933) That this reaction bears a qualitative and quantitative relation to androgenic activity is seen in this series of illustrations, where the intensity of the stain is compared with the clinical picture

Furthermore, he also showed that this stain was present in the cortical cells of the foetus of both sexes It is more marked in the male embryo between the tenth and seventeenth, than it is in the female between the eleventh and fifteenth week This transient 'male phase may be regarded as the latent prototype, activated by the strains and stresses of post natal endocrine life, of the similar stain found in cortical cells of adults suffering from virilism

In point of time this male phase is closely associated with the integration of the foetal endocrine system and probably plays an important part in the determination of the various forms of intersexuality (Broster et al 1938)

BIOCHEMICAL

This Fuchsin stain naturally suggested that the cortical cells were concerned with the elaboration of male hormone or its precursor, and that these substances might be recovered from the urine In 1932 Professor Patterson undertook this biochemical investigation His urinary extracts injected into caponized cockerels by Professor Greenwood in Edinburgh showed the presence of a comb growth producing substance as also that the comb growth was less after adrenalectomy

This biological capon test was extremely laborious and time consuming, and in 1937 Professor Patterson began to exploit and develop the colorimetric test for ketosteroids (1942) About the same time urinary assays were sent to Professors Marrian and Butler in Toronto, who found pregnane triol, a new substance specific to virilism On further analysis of this compound, among other derivates, iso androsterone was recovered In all these investigations the androgen output was decreased after operation In 1945 by further elaboration of this colour reaction Professor Patterson found a test for differentiating between simple and malignant tumours of the cortex (1947)

A general survey of our ketosteroid results based on an analysis up to 1946 may be summarized as follows The normal excretion rate for an adult female ranges from 7–14 mgm /diem and for a male 10–20 mgm /diem In postpubertal virilism the average was 17 6 mgm /diem varying from normal to about 30 mgm /diem with hyperplastic glands ranging from 2 3 to 8 8 grammes The effect of unilateral adrenalectomy is to reduce the ketosteroid output by 50 per cent, and the best clinical results were obtained where the reduction was more than 50 per cent and where the post operative output was lowest This is seen in Table I

In prepubertal virilism the figures are more difficult to analyse, because many of the patients may be young children and the size of the glands each varied between 10–48 grammes In these patients the ketosteroid output may be very high exceeding

100 mgm /diem and rises even higher as they go through puberty, even in spite of unilateral adrenalectomy In seven patients ranging in age from five to twenty two the pre and post operative levels were 37 and 23 mgm /diem, a drop of less than 50 per cent

These biochemical figures have since been improved by subtotal adrenalectomy

TABLE I

Group II Cases (virilism) treated by unilateral adrenalectomy
Relation between clinical result and ketosteroid output

Clinical results	Urinary ketosteroids in mg per day	
	Before operation	After operation
Good Improved Unchanged	17 5 19 1 15 1	6 8 9 9 8 4
Average Keto-steroid output of 41 cases	17 6	8 5

In one patient with feminism submitted to unilateral adrenalectomy the hyper plastic cortex gave a positive fuchsin stain The average preoperative ketosteroid output was slightly reduced by operation from 7 7 mgm a day to 5 6 mgm a day and paradoxically the man improved clinically (1941)

In tumours the androgen excretion may be prodigious An early adenoma the size of a walnut produced 30 mgm /diem a 5 lb carcinoma 400 mgm /diem and a palpable tumour associated with hypoglycaemic fits just under 2 000 mgm /diem

SURGICAL RESULTS

The general result of adrenalectomy is to inhibit or reverse the biological processes which give rise to the syndrome In the tumour group clinical changes are dramatic and complete In hyperplasias they are partial and the benefits of operation vary in their emphasis on particular aspects of the syndrome

Tumour

In tumours the hair falls out somatic and sexual development revert to normal but return when secondary deposits make their appearance It is necessary for these tumours to be diagnosed early by close clinical observation and ketosteroid test when the surgical risk and the possible recurrence rate are at a minimum In all cases the presence of the opposite adrenal should be established

Hyperplasias

The detailed results of the adrenal hyperplasias were published in 1946

In children of the prepubertal group, with adrenal glands the size and the colour of a normal spleen, and with a high androgen output, which rises rapidly during puberty, unilateral adrenalectomy is insufficient We have obtained better results with subtotal adrenalectomy Without the aid of cortisone we have succeeded in removing three quarters of the total amount of adrenal tissue These patients are serious surgical risks, and are prone to develop sudden and unexpected Addisonian crises within three days of operation Several of the older and less marked cases have been satisfied with unilateral adrenalectomy followed by hormone therapy and have grown up useful citizens

The majority of patients belong to the postpubertal group I have now operated on about 130 with no mortality, and a follow up of 88 cases in 1946 showed the following results

TABLE II

Post operative results of 88 patients with post pubertal virilism after unilateral adrenalectomy Average age 26, observed over 16 years (1946)

Results		Fat Cushing Type	With Psychological changes
Good	16	2	5
Improved	45	11	8
Unchanged	10	1	2
Deficient information	14	—	—
Died from other causes	3	—	—
Total	88	14*	15 *

* Included in the total of 88

These young women are often prepared to go to desperate lengths to improve their condition, and an independent analysis stated that 'in well selected cases the opera tion is undoubtedly successful in the amelioration of symptoms While the general health, headache, depression, sterility and sexuality were invariably improved the effect on the hair, periods and obesity was more variable although the majority expressed satisfaction with such benefit as they had received

In 1932, I removed the adrenal from a patient, whom we subsequently regarded as a case of Cushing s type syndrome, the same year in which Cushing published his classical monograph on Basophilism (1932) We were not impressed with this result,

and it was only by encouragement from Harvey Cushing himself that we persevered with more encouraging results The prognosis in this group is not good, and two of our old patients have returned with marked cardiovascular disease from hypertension

The surgical tendency, especially in America, is to be more drastic, and a bilateral or subtotal adrenalectomy is being performed for these cases

In all these groups, until these clinical manifestations can be satisfactorily treated by hormonal means, operation is the treatment of choice

PSYCHOLOGICAL

Disraeli once remarked that man is born to observe but when he falls into psychology he learns nothing This I presume is another way of stating the difference between the objective and subjective forms of study Clifford Allen and I have observed several cases of abnormal behaviour and published one case (1939) with paranoid psychosis that has remained well twelve years after adrenalectomy, except for a mild recrudescence of her psychosis during the menopause The increased incidence of these psychological variations in adrenal work is too important to be dismissed as unproven clinical observations when they promise to lead to a more direct and objective study of the lower reaches of behaviour Tinbergen (1951) quotes Hess who has produced complete instinctive behaviour patterns by direct stimulation of the hypothalamus The work of Dale on synaptic chemical buffers ketosteroid changes after leucotomy and other work suggesting high hormone levels in the central nervous system all show a trend of thought serving to emphasize the importance of biochemical variation with intensification of response and sensitivity to stimuli in the transmission of nervous impulses

HORMONAL TYPES

At the present moment we are engaged in the study of making hormonal assays from blood taken from the living adrenal gland during operation in the different clinical types of the adrenogenital syndrome This work has only just begun and I would like to quote the results of Dr Bush who has undertaken this work He finds that there is a definite difference in the type of steroids secreted in different forms of adrenal hyperfunction

GRAFTING

The surgery of the adrenal cortex will not be complete without some reference to the experimental work of grafting whole glands into patients suffering from Addison s disease In 1946 a case of this kind was published The patient has been under constant supervision now for seven years and both the clinical result and the chemical tests show that she is free from Addison s disease As Dr Gardiner Hill is present at this meeting I am sure you would be interested to hear his independent opinion and observations

In view of the relationship between the pituitary and the suprarenals the question arises whether pituitary grafts could be employed in order that their ACTH might stimulate under-active suprarenals to an increased output of steroids Unfortunately, the pituitary presents special problems of blood supply which add greatly to the

difficulty of successful grafting operations Harris and Jacobsohn (1951) have been successful with pituitary transplants placed in the subarachnoid space near the pituitary stalk They find that in these animal experiments the transplants become revascularized and functional

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Discussion

Chairman R Milnes Walker

G H Bourne Has Mr Broster any knowledge of what the fuchsinophile material is in the suprarenal or any information about its distribution? I had the good fortune some years ago to be able to examine sections of the suprarenals from two cases of adrenal virilism and I found that the number of mitochondria in the suprarenal cells was markedly increased

In view of the recent association of mitochondria with the processes of aerobic respiration in the cell this suggests that there is an increase of metabolic activity in the cortical cells of such cases In view further, of the fact that mitochondria are fuchsinophilic is it possible that the fuchsin staining material described by Mr Broster in the adrenal cortical cells in cases of adrenal virilism may have been mito chondria?

L R Broster I am sorry I do not know the answer

J Groen Have there been amongst your adrenalectomy patients any instances of the syndrome described by Stein and Leventhal in the U S A which has recently attracted the attention of several investigators in the Netherlands This syndrome is considered by some as being caused by the over production of androgenic substances by the ovarian theca cells which have been reported as increased in this condition In fact, the syndrome has been ascribed to *hyperthecosis*

The evidence available at present however is insufficient to warrant this conclusion More particularly one would like to know if the adrenals do not participate in some way in the production of the syndrome

So far as is known no post mortem reports of this condition are available There fore if Mr Broster has ever operated on such cases it would be very valuable if he could give us any information about macroscopic and microscopic appearances of the adrenal gland

L R Broster Dr Vines found follicular cystic degeneration of the ovaries in these cases of hyperthecosis, a syndrome described by D A Du Toit as consisting of poly cystic ovaries menstrual disturbances and hirsutism This syndrome appears to be an early phase of the adreno genital syndrome

I myself wrote a paper on the effect of genetics ovaries and adrenals on hair growth American workers have described an androgenic cell in the ovary

E B Astwood I was interested in the Stein Leventhal syndrome discussed by Dr Groen At our hospital Dr George Mitchell a gynaecologist has found many large pale ovaries in apparently normal healthy women and doubts the existence of this syndrome

H Gardiner Hill I think, if one had to sum up, one would say that we were dealing with three main groups of cases

1 Adrenal pseudohermaphroditism This is a developmental anomaly Twenty years ago we published one of these cases in which Mr Broster had removed an enlarged adrenal The hirsutism improved for a time but the patient never menstruated and ultimately relapsed, presumably *due to hypertrophy of the other adrenal* Gordon Holmes reported a case of adrenal virilism due to an adenoma cured by adrenalectomy

2 Cases of adrenal carcinoma In these the disease is nearly always advanced and in my experience little success attends removal of the growth

3 Cases of pituitary origin i e Cushing s syndrome In these adrenalectomy from the very nature of the condition primarily a pituitary disturbance, cannot be expected to do much good

A Faurbye It is very interesting that 25 per cent of Dr Broster s patients with suprarenal tumours had psychoses

In a survey of 450 female schizophrenic patients we found that 60 per cent had a hypertrichosis and increased urinary ketosteroids as compared with 36 per cent in the surgical wards of the hospital

Hugh Jolly I would like *to suggest that surgery has no place in the treatment of adrenal* hyperplasia My interest is particularly in congenital adrenal hyperplasia on account of a study of sexual precocity which I have made The consequences of congenital adrenal hyperplasia depend on the sex of the child thus in the male sexual precocity becomes evident shortly after birth while the girl is born a female pseudohermaphrodite In both sexes the administration of cortisone reduces the high level of 17 ketosteroids to a more normal figure and with this the symptoms are improved

When I was in America recently I saw a number of Lawson Wilkins patients at Baltimore who are undergoing this form of treatment He was treating six female pseudohermaphrodites between the ages of eight and a half and eighteen and a half *years and breast development had taken place in all while menstruation* had occurred in three It is essential that this form of therapy should be commenced as early in life as possible since if the bone age is already advanced the giving of cortisone immediately brings the sexual age forward to the bone age and therefore may precipitate adolescence Thus in one girl aged six years with a bone age of fourteen years the administration of cortisone caused an immediate puberty

After seeing Wilkins work I feel quite convinced that prolonged cortisone admin*istration to maintain a low level of 17 ketosteroids* and *not surgery* is the correct treatment for these cases Bilateral partial adrenalectomy is a dangerous operation and although it may produce some immediate benefit yet this is temporary and the condition will relapse owing to the recurrence of the adrenal hyperplasia If cortisone is not available, it is far preferable to give no treatment, and if necessary bring some of the female pseudohermaphrodites up as boys rather than subject them to surgery One can only hope that cortisone will soon be available in sufficient quanti*ties in this country* to allow of the treatment of all these children

C J O R Morris I have some interesting data on a case of prepubertal virilism We gave her ACTH and there was no change in her electrolyte balance or eosinophil

count but a marked rise in urinary 17 ketosteroid excretion On administration of cortisone there was a fall in 17 ketosteroid excretion finally down to almost normal levels and a concomitant fall in eosinophil count

I think that the primary action of ACTH is to stimulate the adrenal cortex to break down cholesterol to C19 and C21 compounds which are respectively the precursors of the adrenal androgens and corticoids The conversion of these precursors into the active hormones is not under pituitary control If one or more of the e inter mediate stages in the synthesis of corticoids is defective there will be a decrease in the amount of these hormones secreted although the formation of precursors will be normal On account of the lack of corticoids the secretion of ACTH will be enhanced and accordingly the rate of elaboration of androgens by the adrenal increased, lead ing to the clinical symptoms of virilism

In agreement with this hypothesis we found that this patient was excreting relatively large amounts of adrenal ascorbic acid depleting factor in the urine

G A Overbeek We have been trying to produce Cushing s syndrome in monkeys by the administration of large amounts of various sterols e g DCA cortical extract oestradiol testosterone propionate etc but without results When however we gave the mixture without the oestradiol we got a weight increa e and an erythrocytosis the face went red and the fat distribution seemed to change to that of Cushing s syndrome Testosterone alone is able to cause this effect and the simultaneous adminis tration of oestradiol cures this condition

I should like to ask Mr Broster whether he thinks that there is any possibility of treating some of his cases of adrenal hyperplasia with oestrogens

H Hoagland Huggins in Chicago has done a series of thirty bilateral adrenalectomies for carcinoma usually of the bronchus or prostate In all ca es the men had previously had bilateral orchidectomies The operative risk is not high not higher than for cholecystectomy In all cases there was regression of the primary carcinoma and of the secondaries with relief of pain The patients appeared to be very fit though some of them got recurrences after one year The importance of the right maintenance dose of cortisone and salt must be stressed One patient continued to work whilst he had influenza and got an acute cortical insufficiency The average maintenance dose is 50 mgms corti one and 4 grms of salt a day by mouth at a cost of about 75 cents Many of these patients can live a normal healthy life

L R Broster It has taken us twenty years to reach the limits of pure surgery in adrenal disease and I entirely agree with Dr Jolly that cortisone appears to be the treatment of choice in these rare cases of pre pubertal virilism but we still do not know the limitations of hormone therapy and it may take us some time to find them out In 1936 I myself was disappointed in the partial success of surgery in Cushing s syn drome but I was advised by Harvey Cushing himself to carry on and I feel it has been of benefit to some patients I believe that the operative risk is quite reasonable nowadays and surgery has certainly stimulated a lot of work in this field

In answer to Dr Overbeek we have been able to produce menses and breast growth in some of these cases with oestrogens

Some Recent Developments in the Clinical Use of Cortical Steroids and Corticotropin

by

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THE efficacy of corticotropin and cortisone in the treatment of a great variety of diseases is now so well known as to require no emphasis The diseases which respond favourably are well defined and the metabolic as well as the overdosage effects of therapy are familiar to all It has been clearly established that only a limited number of acute conditions are cured in the sense that the disease process subsides while treatment is being given and it is known that most of the chronic pathological conditions, though they may be completely reversed by therapy promptly relapse when treatment is withdrawn So far there has been no clue as to the mechanism of action nor has the response of these various diseases to treatment led to an understanding of their aetiology Furthermore there is apparently no single feature common to the diseases which are amenable to this form of treatment The most general statement that can be made is that those diseases which respond best are those in which some allergic phenomena seem to be operative The allergic component is apparent in such conditions as asthma sensitivity reactions to drugs serum sickness dermatitis venenata and urticaria It is also generally conceded that hypersensitivity is somehow involved in the pathogenesis of disseminated lupus erythematosus periarteritis pemphigus dermatomyositis and the idiopathic inflammatory diseases of the eye Some sort of allergic response has long been suspected as aetiologic in the rheumatic diseases the acquired haemolytic anaemias and in various diseases of the skin and gastrointestinal tract It is tempting to assume therefore that the disorders which respond to adrenal cortical therapy are those in which an allergic response of tissues is an important factor in pathogenesis

From another aspect the treatment may be regarded as primarily antiphlogistic inflammation whether brought about by infective allergic chemical or physical agents is inhibited or suppressed According to this alternative view the diseases which respond most favourably are those in which the host reaction to the noxious agent is more destructive than the agent itself There are other effects however beyond the apparent anti allergic or anti inflammatory action Fever from most

causes is abolished, the erythrocyte sedimentation rate is reduced malaise is relieved, and some types of pain unrelated to inflammation are relieved The local and systemic manifestations of infections may be largely abolished though the proliferation and spread of the micro organisms are not impeded Many of the reactions of the body to trauma, physical as well as chemical, are inhibited, the lymphoid tissues even when neoplastic are caused temporarily to regress connective tissue growth is suppressed and indeed there are few pathological processes which are not modified in one way or another

During the past year the clinical applications of cortisone and corticotropin therapy have been detailed in numerous contributions to medical literature and several extensive reviews have been prepared (Mote, 1951 Kinsell, 1951, Sprague, 1951) A few of the advances which seem at present to be of especial interest include the introduction of new steroids, especially hydrocortisone and corticosterone the local application of steroids to isolated areas, the use of cortisone in the treatment of adrenal cortical insufficiency and the adrenogenital syndrome, and the development of better preparations of corticotropin for clinical use

CORTICOSTERONE AND HYDROCORTISONE

These steroids have been available for investigation in limited quantities only but they could readily be produced as well as, or instead of cortisone if they proved to be sufficiently promising as therapeutic agents (Carlisle 1952) Both compounds are like cortisone, effective when given orally, but unlike cortisone, they are considered to be normal secretory products of the adrenal cortex Corticosterone shares the properties of both cortisone and desoxycorticosterone (Conn, 1951), but, as judged by the responses of patients suffering from Addison s disease, it is not more than one quarter as active as cortisone and perhaps only one fiftieth as active as desoxycorticosterone in these two respective activities Its use in therapy is therefore restricted to the treatment of adrenal insufficiency and even here it may not be as widely applicable as a mixture of the above mentioned compounds

Hydrocortisone would appear to be the most important hormone secreted by the adrenal cortex Examination of the steroidal metabolic products in human urine (Mason 1950) the steroids in perfusates of the adrenal gland (Hechter *et al* 1951), in adrenal vein blood (Reich Nelson and Zaffaroni 1950), and those formed by adrenal tissue *in vitro* suggest that hydrocortisone rather than cortisone is the chief physiologically active steroidal secretion Its metabolic actions closely resemble those of cortisone In most animal tests it is slightly more active (Ingle) while in man it exhibits all of the properties of cortisone and, in addition, it possesses a somewhat greater capacity to cause water and salt retention (Conn, Louis and Fajans 1951) Though hydrocortisone is a more polar compound than cortisone it is absorbed at a remarkably slow rate when a suspension of the acetate is deposited in the tissues The repeated intramuscular injection of large doses of the acetate gives rise to only a minimal response whereas the free sterol is somewhat more quickly absorbed and after oral administration both compounds are rapidly absorbed and highly effective (Conn *et al*, 1951)

TREATMENT OF ADRENAL INSUFFICIENCY

Since cortisone acetate has become readily available the management of Addison s disease has been rendered simpler and more satisfactory Cortisone is most conveniently given in the form of tablets by mouth, and the dosage required in Addison s disease is small enough to make prolonged treatment economically feasible There seems to be general agreement that if the dose of cortisone be large enough it can be used as the sole form of treatment in this disorder doses of 50 milligrams a day or more not only correct the disturbance in organic metabolism but cause a sufficient effect on salt and water excretion to maintain electrolyte balance A smaller dose may be used if supplemented by extra dietary salt or by desoxycorticosterone acetate (Thorn *et al*, 1951) When this is done the average daily intake need be only about 25 milligrams, usually given in two doses twelve hours apart One may perform special tests to determine adequacy of dosage but for practical management it is often sufficient to be guided by symptoms body weight blood pressure tendency to oedema and perhaps by an occasional determination of the concentration of sodium in the serum Desoxycorticosterone acetate may be given either in the form of sublingual tablets in a dose of 2 to 4 milligrams daily by the implantation of pellets or by the daily subcutaneous injection of an oily solution Just recently, desoxycorticosterone trimethyl acetate has been introduced in the form of an aqueous suspension containing 30 milligrams per cc A single injection of 1 or 2 cc will exert an effect for several months and satisfactory management can be achieved by injections every four weeks (Thorn 1952) On the average 50 milligrams of this preparation monthly with 12½ milligrams of cortisone morning and evening provides satisfactory control Corticosterone though possessing properties both of cortisone and of desoxycorticosterone must be given in large doses and is at present not practicable Furthermore in some cases such large doses are needed that the salt retaining effects become prominent before the other desirable actions are achieved

Hypopituitarism is another condition where substitution therapy is attended by great benefit Small doses of cortisone are remarkably effective and when the appropriate sex hormone and sometimes thyroid are also supplied the patients are maintained in a good state of health The appearance of patients thus treated is still abnormal suggesting that some other essential pituitary factor is still lacking

Bilateral adrenocortical hyperplasia It is now possible for the first time to suppress the excessive androgen secretion of patients suffering from the adrenogenital syndrome In adults doses of 100 milligrams per day or less and in children daily doses of 25 milligrams suppress to normal or below the excessive urinary excretion of keto steroids Correspondingly there is clinical evidence of decreased androgen action and ovarian function may become normal (Wilkins *et al* 1951 Forbes and Albright 1951) As these patients may exhibit mild or severe manifestations of deficient adrenal steroid secretion the treatment provides substitution therapy as well

Bilateral adrenalectomy This operation has recently been performed for the treatment of Cushing s syndrome hypertension and carcinoma of the prostate (Huggins and Bergenstal 1951) The procedure presupposes that ensuing adrenal insufficiency can be satisfactorily controlled by substitution therapy In the case of Cushing s syndrome the procedure may be life saving and in this instance a subtotal adrenalectomy is

apparently feasible The small remnant of adrenal tissue may sometimes regenerate and lead to recurrence, sometimes it does not survive and Addison s disease supervenes, but in a significant proportion of cases normal adrenal function results Though bilateral adrenalectomy has been carried out supplementary to orchidectomy as palliative treatment for carcinoma of the prostate, experience has not yet been sufficient to show whether such a procedure is beneficial Therapy with cortisone for the induced adrenal insufficiency may in part be responsible for the symptomatic benefit for some patients with prostatic carcinoma experience symptomatic relief from cortisone therapy alone The procedure is adopted because it is assumed that the adrenal cortex produces an androgen which might promote the growth of the prostatic neoplasm This assumption is open to question for, though the adrenal cortex secretes substances which give rise to ketosteroids in the urine, there is reason to believe that the adrenal cortical secretions are not androgenic under normal circumstances

Some encouraging results have been obtained from bilateral adrenalectomy in patients suffering from hypertensive cardiovascular disease (Wolferth *et al*, 1951) In this instance many of the patients are in a critical condition at the time of operation and substitution therapy and postoperative management may be beset with difficulties Advanced renal failure appears to be one contra indication to this form of treatment because of increased hazard of the procedure and the added problem of subsequent management

The treatment of patients undergoing adrenalectomy involves the administration of cortisone in doses of about 300 milligrams daily parenterally for two to three days prior to operation with continued dosage on the day of, and for a few days after, the surgical procedure Intramuscular administration in this way will ensure a steady, slow absorption over many days It is advisable to provide this depot because the patient may be unable to take the hormone by mouth, and at present there is no simple way of obtaining a rapid response to cortisone except by the oral route This emphasizes the need for a preparation of cortisone which can be safely given intravenously Subsequent management of these patients is similar to that in Addison s disease It is believed that patients with hypertension should be maintained on a minimal dosage of salt and desoxycorticosterone substitution being provided in so far as possible by cortisone alone Conversely some have claimed that, in the management of patients operated upon for carcinoma of the prostate the dosage of cortisone should be kept down in order to obtain a minimal excretion of urinary ketosteroids It should be recalled however that cortisone is not androgenic and there would seem to be no contra indication to its use in this condition

Preoperative therapy with cortisone has also facilitated surgical treatment of pituitary tumours Here of course corticotropin could be used instead, corticotropin having the advantage of rapid action when cortisone cannot be given by mouth

Substitution therapy in Addison s disease and following total adrenalectomy can not be regarded as entirely effective One has no knowledge of the hormone requirements during stress and though one may increase the dosage by some arbitrary amount in the event of infection, trauma exposure or starvation the adrenalectomized patient lives a precarious existence Should the therapy be inadvertently omitted for a few days or should it not be promptly increased at times of illness or stress, sudden death may occur

LOCAL STEROID THERAPY

Probably the most ideal conditions for this new form of treatment are those which can be treated by the direct application of cortisone or hydrocortisone to the localized area of disease Here the quantity of steroid needed is small and intensive therapy can be given with no fear of systemic overdosage effects Local therapy finds its widest application in various inflammatory diseases of the eye (Olson *et al* 1950) The steroid is applied as a solution or suspension into the conjunctival sac at frequent intervals or by the subconjunctival instillation of a small deposit of the crystals The hormone appears to penetrate the deeper tissues of the eye for not only do superficial inflammations, such as conjunctivitis respond but also certain inflammatory reactions involving parts of the uveal tract More extensive inflammatory processes require systemic therapy however

Skin lesions have been treated by the local application of various ointments containing the sterols, but thus far the effects have not been great Generalized dermatological conditions are more efficiently treated by systemic therapy but there sometimes remain limited areas of the body showing an incomplete response which seem to benefit from additional local applications The local instillation of cortisone or hydrocortisone into affected joints has been shown to be effective Here hydrocortisone appears to be the more effective (Hollander *et al* 1951) and this may be related to the slower absorption of hydrocortisone Apparently both rheumatoid and osteoarthritic joints show improvement and the treatment has also been applied with success in gout and in bursitis The duration of action of a single injection varies widely from a few days to several weeks In this case too local therapy of one or two unusually troublesome joints can supplement systemic therapy

Local therapy of asthma by the inhalation of cortisone in the form of an aerosol has been successful (Gelfand 1951) and consideration has been given to local application to such areas as the nasal mucosa as well as to the bladder the vagina and the colon

CLINICAL USE OF PURIFIED CORTICOTROPIN PREPARATIONS

Until very recently routine therapy with corticotropin was carried out by the intramuscular or subcutaneous injection of aqueous solutions of crude extracts at six to eight hour intervals The potency of commercial preparations varied from less than one to two or three units per milligram The remarkable observation was made that the continuous infusion of crude corticotropin intravenously induced a much more marked effect than a similar amount given intramuscularly (Gordon 1950) Some preparations were as much as twenty times as effective by the intravenous route When purer preparations of corticotropin were used clinically it was immediately apparent that they were more effective than would have been anticipated on the basis of their biological activity (Rosenberg *et al* 1951) and when extracts (Astwood Raben Payne and Grady 1951) containing 80 to 100 units per milligram were employed a very striking enhancement in clinical effectiveness was apparent (Raben Rosenberg Westermeyer and Astwood 1952) The intravenous infusion of these purer products did not greatly enhance effectiveness and thus in effect purification accomplished the same result as continuous intravenous infusion of crude

material The reason for this added effectiveness is not entirely clear, but one explanation involves the local destruction of the active principle in the crude extracts at the site of injection It is known that standard preparations of corticotropin contain proteolytic enzymes and that these extracts lose activity in neutral solution (Adams and Smith, 1951) It would seem likely, therefore, that some destructive agent might account for the inefficiency of the crude solutions Presumably the injected material would approach the pH of the body fluids before all of it is absorbed into the circulation Furthermore it has been claimed that many of the cruder preparations are destroyed on admixture with blood, while purified corticotropin is not, suggesting that there is an additional factor present in the blood acting in conjunction with something in the crude extracts which promotes the destruction of corticotropin (Pincus Hopkins, and Hechter 1952)

These findings might explain the earlier failures when attempts were made to develop efficient long acting or depot type corticotropin preparations Perhaps if the extract were efficiently held at the site of injection it might suffer still greater destruction and be less effective than if it were given in simple aqueous solution Purified corticotropin on the other hand, lends itself well to depot type therapy Upon admixture with any one of several media designed to retard absorption, the material appears to become more efficient so that smaller daily doses are required Gelatin is one suitable retarding medium for human use and a satisfactory product contains 40 units (about 0 5 milligram) of purified corticotropin in 10 per cent gelatin solution Most patients can be treated by once daily injections of 0 2 to 1 0 cc of this solution subcutaneously the dose being related to the severity of the condition under treatment This added effectiveness which attends purification and incorporation into retarding media extends the available supplies of pituitary glands and should reduce the cost of therapy An average daily dose of 0 3 milligram would be derived from about 15 milligrams of crude extract which in turn would be obtained from 120 milligrams of powdered anterior pituitary—the approximate dry weight of one hog hypophysis

RELATIVE MERITS OF CORTISONE AND CORTICOTROPIN

When it became known that cortisone is effective when given by mouth it seemed likely that it would soon supplant corticotropin in clinical practice oral therapy was more convenient than parenteral, especially as the latter involved intramuscular injections of crude material at six hour intervals Cortisone was found to have the added advantage of inducing less retention of water and salt than corticotropin and was preferable for those patients with impaired circulation or diminished renal functional capacity It soon became apparent, however, that some conditions did not respond as well to cortisone as to corticotropin severe asthmatic attacks could not be interrupted even with as much as one half gram of cortisone daily and comparably large doses were needed in other severe conditions such as pemphigus periarteritis, disseminated lupus and acquired haemolytic anaemia Individual cases of rheumatoid arthritis and other less severe conditions were also found to respond more quickly and more completely to corticotropin When purified corticotropin became available in a form permitting delayed absorption therapy could be provided by a single

subcutaneous injection every twenty four to forty eight hours, patients could administer the small volume of 0 2 to 1 0 cc themselves using a fine hypodermic needle and a steady effect could thereby be achieved The large differential of convenience was thus greatly reduced and some patients prefer corticotropin taken in this way to cortisone taken throughout the day and night by mouth

It seems likely, too that for some years to come corticotropin therapy will be less expensive than treatment with adrenal cortical steroids A minimal daily dose of 100–200 milligrams of cortisone now costs the patient about £1 per day and it seems unlikely that this cost will be greatly reduced in the immediate future It should be possible however to reduce greatly the cost of corticotropin One pound of pituitary powder at the current market price of £40 should provide enough purified corticotropin for four thousand treatment days If the cost of preparation did not greatly exceed the cost of the raw material the expense to the patient should be about one shilling per day Currently in the United States therapy with purified corticotropin in gelatin costs an average of $1 00 per day and it is anticipated that this figure will soon be reduced

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Clinical Responses as Illustrated by Treatment of the Rheumatic Diseases

by

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OWING to the need for brevity it is quite impossible to discuss the clinical responses to cortisone of all the large number of conditions for which it has been used The reason why acquired but not congenital acholuric jaundice is controlled the ethics of the use of cortisone in acute leukaemia and its mode of action and its theoretical dangers of spreading tuberculosis when u ed in sarcoidosis are all most interesting questions There is no time even to touch on these I will confine myself to the principles governing the practical uses of cortisone illustrating them by reference especially to the rheumatic group of diseases and then comment on clinical resistance to these hormones

BASIC CONDITIONS GOVERNING THERAPY

Cortisone as we have heard has a peripheral effect possibly after its alteration in the body to hydrocortisone or some similar compound and also a central effect in combating shock It controls hyper ergy and reduces the formation of new scar tissue Its action as we well know is only temporary In order to give a clear answer as to the value of cortisone in any particular case a consideration of both the seed and the soil is necessary By seed is denoted the various physiological effects of cortisone and by the soil the patient We must consider the disease under treatment the stage of the disease and its normal natural history and also any other disease from which the patient may suffer and which may be affected by undesirable side effects of cortisone—peptic ulceration diabetes, tuberculosis or psychosis Hyper ergy may kill annoy or protect and the same may be said of scar tissue The effect of cortisone on these processes may therefore be either beneficial or detrimental Sweeping statements as to the value of cortisone and ACTH are not warranted and each case requires consideration on its individual merits

The likening of cortisone to an asbestos suit, which temporarily protects but neither puts out the flame nor deals with the ashes is well known Another useful simile is the recharging of an accumulator as this brings out the necessity for keeping the headlights turned off if the recharging is to produce the desired effect

EXAMPLES OF USE

In ophthalmology cortisone may save sight by holding up a hyper ergic inflam mation which would be followed by vascularization, but it has been proved to prevent or delay healing In burns it may be of value in the shock phase and later to prevent scar formation but use of antibiotics and antiseptics are then doubly important In generalized lupus large quantities for a long time are essential if treatment is to be worth while, yet in a difficult case of Addison's disease a very small quantity used physiologically, together with DCA will make all the difference to adequate control

In rheumatic fever, ACTH and cortisone are said by some to have a similar action clinically to the salicylates—while removing joint symptoms fever and tachycardia, cardiac lesions and nodules have been known to develop

In rheumatoid arthritis symptoms are usually either controlled or much modified temporarily by these hormones and on these grounds they have been proved to be of value (1) to control an acute onset or exacerbation due to a temporary or removable cause (2) to assist in rehabilitation of the more chronic case that is just failing to make the grade of working or caring for himself—with physical treatment encourage ment and modification of the mode of living or working, many such cases can be helped materially by a short course of treatment, (3) for use, *when necessary*, after surgery in active rheumatoid arthritis—it should be remembered that in some cases the shock of the knife seems to *reduce* the activity of the disease, (4) to cover mani pulative treatment to straighten flexed knees and reduce other contractures—we are at the moment engaged on a series of such cases under the auspices of the Nuffield Foundation and the Medical Research Council, and the immediate results appear to be encouraging in so far as the patient has less reaction and pain after the manipula tion

Work is at present being carried out here in a combined operation with the Bath and Bristol orthopaedists to see whether cortisone will improve the results of cup arthroplasty of the hip It is too early to assess the results but so far there have at least been no untoward results

In long term treatment the value is much more debatable, and the tendency towards *peptic ulceration diabetes tuberculosis thrombosis or psychosis is a definite* contra indication The large majority of cases relapse when treatment is stopped and when more than 50 or at the most 75 mg of cortisone per day is required to pro duce a reasonable control of symptoms, complications are such that prolongation of treatment is usually not advisable There is also a danger of taking the brake off' with cortisone and hence actually increasing joint damage

It is of interest that ACTH like effects have been claimed for nitrogen mustard and aminoptyrin both presumably acting like the hypoglycaemia we have been in vestigating here as stressors stimulating the pituitary adrenal axis They also are said to cause an eosinopenia and an increase in 17 ketosteroid excretion

RESISTANCE TO TREATMENT

In speaking of the effect of ACTH and cortisone on rheumatoid arthritis I said the symptoms could *usually* be controlled temporarily but this is not invariably the case

and clinical response does not always coincide with laboratory responses especially with regard to the depression of the circulating eosinophils

Our first evidence of lack of correlation was obtained in our attempts to stimulate the pituitary with hypoglycaemia and here we found an excellent eosinophil response almost *invariably* but only 44 per cent showed a definite clinical improvement

Since then we have observed two cases that have been completely and one almost resistant clinically to ACTH in large dosage They had neither signs nor symptoms of adrenal failure Kepple tests were normal and other biochemical responses appeared normal One case was also completely resistant to cortisone

A study of such failures may help to elucidate further the mechanism of action in the successes In the enzymology of the tissues and their control by other organs possibly the spleen and liver the answer may rest I would like to cite two examples

G B male aged twenty two had suffered from severe and very active rheumatoid arthritis for four years His E S R was 113 mm (Westergren) He was completely achlorhydric During control injections and with 100 mg ACTH there was no change in his clinical condition or in his excretion of 17 ketosteroids or oxysteroids With 200 mg cortisone however there was a good clinical response a drop in the urinary ketosteroids and a possibly significant increase in the cortins After this test a period of four months elapsed during which time a splenectomy was performed He then had another period of control injections and 400 mg ACTH per day with no clinical change but a slight increase in cortins on the fifth day As it was felt that non absorption of ACTH might be the cause of the absence of clinical response he was then given ACTH intravenously—20 mg on the first day by drip over an eight hour period and 100 mg on the second day again without response

The uric acid creatinine ratio in the urine showed no significant change at any time during the experiment Throughout there was a satisfactory Thorn test—with the intravenous ACTH the drop in eosinophils being 92 per cent Neither ascorbic acid nor small doses of thyroxin affected the clinical result

T S a male aged thirty seven had suffered from active rheumatoid arthritis for three years E S R 57 mm (Westergren) This patient again was achlor hydric With 200 mg ACTH per day there was no response clinically or in urinary steroid excretion With 400 mg again there was no clinical response but a slight increase in the 17 ketosteroids After a rest period he was then given 500 mg cortisone per day with no clinical improvement at all but there was a doubtfully significant decrease in 17 ketosteroids and increase in urinary cortins Intravenous ACTH 20 and then 100 mg again was without effect As in the previous case there was no significant variation in the uric acid creatinine ratio The eosinophil respon e throughout was satisfactory averaging about 70 per cent decrease when it was examined Ascorbic acid and thyroxin again failed to potentiate the ACTH

By courtesy of Dr Reiss the thyroid index was estimated with radio iodine and found to be 2 6 i e within the low range of normality

This patient was then given two intra articular injections of 50 mg cortisone and then two injections into an inflamed wrist joint of 50 mg hydrocortisone acetate with no general or local improvement or eosinopenic response

The absence of clinical response is perhaps not surprising since cortisone and ACTH are not specifics for rheumatoid arthritis and similar exceptions occur to

the rule that pregnancy temporarily reduces the activity of this disease—in a few cases it appears even to aggravate the condition

The absence of correlation between the clinical response and eosinophil depression might actually be expected, in view of Professor Young's recent work on separate adrenal weight increasing and ascorbic acid depleting fractions of ACTH

To summarize the position one may state that, though there is so much we do not know about the fundamental action of cortisone and ACTH, we are in this country formulating an opinion of its clinical value The enthusiastic swing has returned to the vertical and there are signs in some quarters of an over swing in the other direction The therapeutic value of cortisone in ophthalmology is acknowledged even by the most sceptical, and it is finding its place in the treatment of many other conditions, not as a cure, but as a useful tool We feel, however, that its uncontrolled use may well be a danger, especially in suppression of symptoms of other disease and infection In long term treatment, its side effects including those on wound healing and thrombosis, and its withdrawal symptoms and their psychological con sequences may become a serious menace

Discussion

ON PAPERS BY (1) ASTWOOD (2) KERSLEY

Chairman C B Perry

Dr P Ellman I should like to congratulate both Dr Astwood and Dr Kersley on their excellent papers and to ask their advice on a case of rheumatoid arthritis with adrenal insufficiency Incidentally only five other cases of this combination have been reported in the literature three in France in 1947 and two in America in 1950 and 1951 The patient was a woman aged fifty three with a history of acute poly arthritis in 1947 which was successfully dealt with by six weeks routine treatment in bed and a course of gold injections In 1949 a recurrence of the condition was treated in the same way In 1950 she developed lobar pneumonia from which she made a good recovery but she now has dizziness attacks of severe vomiting at least four times a week, and has bronzing of the face chest and arms In September 1951 a diagnosis was made of adrenal insufficiency with rheumatoid disease

We investigated the different effects of treatment with ACTH, cortisone DCA and the combined effects of cortisone and DCA implant in relation to the patient's eosinophil count right hand grip, E S R, weight, water diuresis blood pressure, serum electrolytes urinary chlorides, urinary 17 ketosteroids, skin colour, and the amount of wrist pain and swelling

Treatment with 45 mg of ACTH a day caused diminution of the wrist pain and swelling with improvement of the right hand grip and disappearance of the epitrochlear gland The eosinophil count was lowered but the E S R and body weight were unchanged as was the skin colour There was a feeling of well being and the diastolic blood pressure rose from 60 to 80 mm of mercury The serum chloride

increased to 550 mg per cent while the urinary chlorides fell to less than 10 mg per cent there being no change in the water diuresis or 17 ketosteroid excretion

Cortisone in doses of 25 mg a day for three days, followed by 50 mg a day for ten days and finally 75 mg a day for a further ten days caused almost complete relief of the joint symptoms with a corresponding improvement in the right hand grip and disappearance of the epitrochlear gland There was marked euphoria, the diastolic blood pressure was 80 mm of mercury and the E S R fell to 10 mm in one hour The skin became lighter in colour but there was no increase in weight The eosinophil count rose to 200 per cu mm the serum sodium to 346 mg per cent and the serum chloride to 562 mg per cent while there was no increase in the urinary chloride which remained below 10 mg per cent The maximum water diuresis was 1,080 ml and the patient felt much stronger

On reducing the cortisone dosage to 25 mg a day by reason of shortage of supply the pain recurred and on withdrawal of the cortisone altogether the patient became depressed, lassitude and fatigue appeared on the second day, and by the fourth day the epitrochlear gland was again palpable

Dr Bishop then suggested giving 5 mg of DCA and 6 grs of salt per day The response was favourable as far as the adrenal symptoms were concerned so an implant of 300 mg of DCA (approximately equal to a daily dose of 1 5 mg) was carried out The wrist symptoms increased and the right hand grip became weaker whilst the epitrochlear gland became palpable The patient was depressed and there was a slight fall in weight The E S R rose to 20 and the blood pressure at first falling to 60 mm diastolic soon rose again to 80 mm The urinary chlorides remained below 10 and the total diuresis at no time exceeded the nocturnal specimen

After the DCA implant the patient was discharged for two weeks She was re admitted as soon as further cortisone supplies became available The response to DCA as far as the joints are concerned was very disappointing but it was thought that a smaller dose of cortisone would now be needed to maintain her joints in comfort and to restore the water diuresis to its previous level On readmission her wrists were puffy the epitrochlear glands were large and painful the right grip was 30 mm Hg The patient was depressed and fatigued The skin colour was good

We then studied the combined effects of cortisone and DCA implant 25 mg of cortisone were used by injection for eleven days and followed ubsequently by oral doses not exceeding 50 mg a day Improvement in wrist joints occurred on the eighth day of this regime and was associated with a feeling of well being The right grip rose after initial fluctuations to 60 mm Hg Gradual rise of the diastolic pressure to an average of 90 mm Hg became apparent in the second week This was associated with some sodium and chloride retention and a further fall in urinary chlorides The water diuresis became modified and nearly normal towards the end of the 50 mg period The E S R fell from 20 to 3 The eosinophils gradually climbed from 10 to 56 The weight remained constant and there was no change in the daily excretion of 17 ketosteroids The haemoglobin level initially 12 5 grams reached 14 9 grams at the end of the course

The patient was discharged on a maintenance dose of 25 mg of cortisone per day by mouth She continues on this dose and remains extremely well

She is a contact of her husband who has open tuberculosis and her own Mantoux is positive although she has no active disease I should like to ask

1 Are we justified in continuing the treatment in spite of the tuberculosis risk?

2 Are we likely to get more of these combined cases with more efficient treatment of Addison s disease?

3 What is the prognosis in this case?

G D Kersley In reply to Dr Ellman s inquiry about the treatment of his T B contact with rheumatoid arthritis, if the patient is controlled on 25 mg of cortisone a day and she has no obvious lesions in her lung I think you can carry on with the treatment Dr Robinson has shown that one has to give dosage of the order of at least 200 mg a day before there is any risk of the disastrous spread of infection that sometimes occurs At this low dosage level I feel that the effect will be entirely beneficial

L Weissbecker I agree with Dr Kersley

We have found that from 6–8 per cent of rheumatoid arthritis patients fail to react to either cortisone or to ACTH Could this be due to a disturbance in intermediary steroid metabolism? In thirty cases of rheumatoid arthritis we found that they had *a raised urinary ketosteroid level and a reduced urinary glucocorticoid level* The administration of DCA to normals caused the 17 ketosteroids to fall and the urinary glucocorticoids to rise, this may be an example of the conversion of DCA to a gluco corticoid Possibly the trouble with rheumatoid cases that do not respond to cortisone is that they are unable to 'interconvert their steroid hormones, particularly in the case of a desoxy oxy transformation

A similar inability to carry out this conversion has been found in Addison's disease *and in several cases of ulcerative colitis*

G Sala In the course of the last two years, Dr Ballabio and I have treated one hundred rheumatic patients with cortisone and we are rather optimistic about the results Most rheumatoid arthritis patients respond well to a daily dose of 50–75 mg of cortisone and at this dosage level we do not usually get side effects In rheumatic fever cortisone appears to have a beneficial effect on the general condition on the arthritic symptoms and on the pericardial and myocardial conditions The effect on endocarditis is still under study Therefore we think that cortisone treatment may be instituted in every case of rheumatic fever and in most cases of rheumatoid arthritis

H F West I would like to tell you of two patients with rheumatoid disease whom we treated with cortisone for more than a year

The first was a man aged forty eight After six months treatment, averaging 50 mg daily, he developed for the first time symptoms of a duodenal ulcer At the end of a further six months he had a severe haematemesis This was treated in the usual way, his cortisone being now given by the intramuscular route instead of the oral A month later the ulcer was removed It was of medium size, deep and of long standing The thrombus in the large vessel which had bled was organized and already partly recanalized *apparently unaffected by the cortisone*

The second patient a man of twenty eight with unusually severe and crippling rheumatoid disease, had a liver biopsy which showed no amyloid substance Six months later he began taking cortisone After a year on an average daily dose (by

mouth) of 75 mg (and a high protein diet) his liver weighed 6,470 grams and was 95 per cent amyloid

C Bruce Perry Dr West, how did you manage to learn the weight of this patient's liver?

H F West That is another story After a year's treatment he felt well in himself and chose a supper of fried potatoes (not usually available) to celebrate the occasion Two weeks later he became ill during the night He vomited and had some abdominal pain His blood pressure fell from a somewhat raised level to about 70/40 His blood electrolytes were normal at this time In spite of additional cortisone and a blood transfusion his blood pressure did not recover and he died on the fourth day in the *early* stages of uraemia His adrenals were replaced almost entirely by amyloid substance The kidneys contained very little deposit indeed

Now I expect I had better tell you how we obtained the ulcer from the first case He recovered from his haematemesis but stopped taking cortisone on the twenty fifth day after the haemorrhage On the thirtieth day at noon without premonitory symptoms of adrenal insufficiency he fainted regained consciousness for a minute vomited and died A post mortem was performed within four hours Nothing was found to explain his sudden death Professor Yoffey has since reported the adrenal cortex to be histologically normal Sections of the heart showed marked myocardial fibrosis

P P Lambert As Dr Kersley has reported on the administration of insulin in patients with rheumatoid arthritis I only wish to say that two years ago we performed some similar trials Our patients received no more than 120 mg of ACTH in three days Thereafter we started immediately with insulin in order to prolong the effects of these small doses of ACTH Insulin was given intravenously 0 1 unit per kg of body weight three times a day at the proper time before meals

Intensity of hypoglycaemia and eosinopenic effects were tested every morning—insulin was continued for about three weeks

Of the nine patients treated two had a rapid relapse one of them quickly developing severe urticaria one patient who had relapsed very rapidly after a three days period of ACTH without insulin was successfully treated fifteen days later with ACTH followed by insulin In the six other patients there was no relapse for sixteen to twenty days after suppression of ACTH Some clinical benefit could be observed during the insulin period for instance the time taken to walk a definite distance decreased or remained constant sedimentation rate and plasma fibrinogen remained low for a few days then went up without reaching the pretreatment level In most cases after three weeks of treatment insensibility to insulin appeared which was demonstrated by a very low response in the eosinopenic test and this seemed clearly associated with the clinical relapse

We do not think that hypoglycaemia is a good treatment for rheumatoid arthritis We merely feel that it helped to avoid the severe relapses frequently observed after short periods of treatment with ACTH

P A Bastenie Dr West's patient died of hypoglycaemia

C B Perry What is the position of ACTH and cortisone in regard to sarcoid?

J M Yoffey What is the present attitude towards the effects of ACTH and steroids

on leukaemic conditions, and also in anaemia? Our own bone marrow work in guinea pigs seems to suggest a definite erythropoietic action

E B Astwood I have no authority to speak on blood disease but I have seen several of Dr Dameshek s cases Cases of acute lymphatic leukaemia in children respond temporarily quite well but in adults leukaemia seems to be aggravated by cortisone Cases of acquired haemolytic anaemia seem to do very well I have only seen two cases of sarcoid and in neither did it have any effect However other observers have obtained more promising results particularly in sarcoid disease of the lung

In connection with the resistance of some patients to ACTH, Dr Thorn says that he has seen many cases resistant to commercial preparations but all of them were responsive when purified preparations were used

As regards Dr West s 100–200 mg of cortisone a day for a year—it would surely cause marked metabolic disturbances including presumably atrophy of the supra renal cortex Dr West has shown dramatically one of the greatest disadvantages of cortisone that is the induced adrenocortical insufficiency

Hugh Jolly In the United States both ACTH and cortisone are used in the treatment of acute leukaemia in children and my experience was that individuals varied in their preference as to which of these drugs was used However in almost all centres one or other of these drugs is given and nowhere did I find the attitude that the children should be permitted to die without attempted therapy Both in terms of the blood picture, and the general clinical condition it is usual to obtain an immediate re sponse which may last for a few weeks and sometimes months I was particularly impressed by the immediate disappearance of bone pains, which are often severe with this form of therapy

Once the condition relapses it is usual to turn to aminopterin and when this drug loses its effect to turn back to ACTH or cortisone The longest survival that I heard of on this regime was three years

J J R Duthie In rheumatoid arthritis the dose of cortisone which is required to suppress the inflammatory process frequently causes signs of hyperadrenalism This would suggest that the hormone is not metabolized by the tissue on which it acts as signs of hyperadrenalism appear with equal frequency in cases with many joints affected and those with minimal signs of activity It may be that the hormone acts in stages or that it is metabolites which are active in suppressing inflammation

E B Astwood Direct observation refutes the idea that large areas of inflammation need large doses of hormone The hormone does not appear to be used up as it works and there does not seem to be an increased utilization of the hormone by the diseased tissues Severely ill patients exhibit overdosage effects with the same quantity of hormone as do normal persons

F Verzar I was very impressed by Professor Cameron, who seems to have come to the same answer as the physiologists that it is a change in the metabolism of the tissue cells that occurs after cortisone therapy What is this change? Is it that the carbohydrate metabolism is altered and that this causes secondary changes in the mineral balance of potassium and sodium?

How do large doses of cortisone relieve the pain in rheumatoid arthritis? It may be that in the muscle etc there is an enzyme disturbance of carbohydrate metabolism

so that large amounts of potassium accumulate outside the cells If potassium is Lewis s pain substance its presence intramuscularly may be the cause of the pain Large doses of cortisone may restore the enzyme system to normal and cause the excessive extracellular potassium to return to the cells This is of course, pure speculation

H Hoagland Should not DCA be pain relieving in this case? When it is injected into a joint?

F Verzar No DCA might only work after its relatively slow transformation into 11 oxy compounds

H Hoagland I have seen some very rapid results with DCA

H J Robinson Two years ago it was fashionable to publish the good effects of corti sone or ACTH therapy, now it is becoming more fashionable to publish the ill effects of therapy The latter is partially due to the fact that it is no longer news to publish on the therapeutic value of cortisone in rheumatoid arthritis etc However it is news to point out that one patient in a thousand develops a prolonged clotting time I believe that eventually a happy medium will be reached and cortisone and ACTH will enjoy wide usage in clinical medicine

A G Baikie Dameshek s suggestion of increased erythropoiesis due to cortisone or ACTH is apparently based on work done on acquired haemolytic anaemia Wintrobe and others have been unable to show any effect of cortisone or ACTH in cases of aplastic anaemia etc There is no evidence of these hormones having a significant erythropoietic effect except in cases where there is an antigen antibody reaction In these cases there is often a dramatic response

J M Yoffey There is always a mild hypochromic anaemia in rheumatoid arthritis and I believe that these hormones have a marked effect on that

J J R Duthie That is true but intravenous iron may also correct the anaemia in some of these cases One can get a change of haemoglobin from 60–90 per cent without altering the degree of haemoglobination of the individual cells In one or two cases of rheumatoid arthritis with a severe degree of anaemia we have found the Coombs test to be positive and the possibility exists that the anaemia is due to in creased destruction of red blood cells

L Weissbecker In Freiburg we have noticed a reticulocytosis of from 30–60 reticu locyes per 1 000 red blood cells in cases of rheumatoid arthritis commencing treat ment with cortisone or ACTH We have also noticed a thrombocytosis

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